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Volume 40 *Number 12*

Phytopathology

AN INTERNATIONAL JOURNAL

December 1950

Official Organ of

The American Phytopathological Society

$7.75 per year, Canada $7.50 per year, United States and dependencies $8.00 per year, elsewhere

Office of Publication

Corner North Queen Street and McGovern Avenue, Lancaster, Pennsylvania

Entered at the postoffice in Lancaster, Pennsylvania, as second-class matter. Acceptance for mailing at the special rate of postage provided for in paragraph (d-2) Section 34.40, P. L. & R. of 1948. Authorized May 12, 1921.

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LOOSE KERNEL SMUT OF JOHNSON GRASS

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(Accepted for publication May 1, 1950)

INTRODUCTION

Johnson grass, *Sorghum halepense* (L.) Pers., is widely distributed over the agricultural areas of the southern half of the United States. Although it is an important source of pasture and hay, it is generally classed as a noxious perennial weed, largely because of its persistence. Its manner of spreading by means of underground rhizomes makes its eradication difficult.

Smut on Johnson grass has been observed for many years. Among the herbarium specimens at the Plant Industry Station, Beltsville, Maryland, are some collected as early as 1903. Collections have been made in 12 States and four foreign countries. Frequently patches of Johnson grass are observed in which practically all of the panicles are smutted. The causal organism, however, has received little study, probably because of minor appreciation of the economic importance of the host plant.

Many of the herbarium collections of smut on Johnson grass in the Plant Industry Station herbarium are labeled *Sphacelotheca cruenta* (Kuehn) Potter or *S. sorghi* (Lk.) Clint. Specimens collected by Johnston et al. (3) in Kansas in 1935 and in Oklahoma in 1936 (4) were identified by George L. Zundel as *Sphacelotheca holci* Jackson. This species was collected on cultivated sorghum in Venezuela in 1934 (2). Johnson-grass smut received from the Union of South Africa in 1926 also proved to be this same species (3). Other specimens were received from Cuba, Mexico, and Palestine.

In 1937 Rodenhiser (5), using as inoculum smut from Johnson grass collected near Sacaton, Arizona, obtained an average of 15.7 per cent infection in Sudan grass, 43.3 per cent in Johnson grass, and 80 per cent in kafir × feterita (K.B. 2510). Reed kafir, Dwarf Yellow milo, and Pierce kaferita appeared immune. On the basis of these results, he suggested that this smut might be a new physiologic race of *Sphacelotheca cruenta*.

In 1938, Johnston et al. (4) reported that spores of Johnson-grass smut were larger than spores of *Sphacelotheca cruenta*, and caused more severe stunting, more profuse tillering, and more branching of infected sorghum plants. *S. holci* also failed to infect some varieties that are very susceptible to *S. cruenta*. They concluded that the two smuts are only distantly related. Therefore, in this paper, the smut on Johnson grass will be referred to as *S. holci*.

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The susceptibility of certain sorghum varieties to *Sphacelotheca holci* made it appear to be a potential threat to sorghum culture. Sorghum fields are often infested with, or surrounded by, smutted Johnson grass. It seemed desirable, therefore, to study its host range, its method of infection and spread, and certain other phases of its pathogenicity that might affect its importance as a sorghum pathogen.

MATERIAL AND METHODS

Smutted heads of Johnson grass were obtained each spring or summer from areas where such infected plants are common. Infected plants also were obtained for transplanting.[2] Healthy plants of Johnson grass were transplanted from a roadside area near Lanham, Maryland, fully 100 miles north of the heavily infested Johnson-grass range. Seed of Johnson grass was obtained through the Soil Conservation Service of San Antonio, Texas, and later from plants grown at the Plant Industry Station. All seed used in these studies was surface-sterilized in a 1–240 formaldehyde solution for 30 min., washed in running water for 5 min., and then dried.

In temperature studies in the greenhouse, inoculated seed was planted in steamed soil in metal boxes $8 \times 4 \times 2$ in. The boxes were placed in constant temperature chambers until after the plants had emerged, after which the seedlings were transplanted to 6-in. pots or to greenhouse beds.

Other studies were carried out in pots, greenhouse beds, and field plots.

EXPERIMENTAL RESULTS

Relation of Temperature to Seedling Infection

All attempts to determine what effect, if any, temperature has on infection in Johnson grass from seed inoculation were unsuccessful because of poor stands, especially at the lower temperatures, and failure to obtain infection. Seeds of Johnson grass, inoculated with fresh viable spores of *S. holci*, were planted at each of six temperatures—15°, 20°, 25°, 30°, 35°, and 40° C. Seeds with and without hulls were used at all six temperatures with negative results. In addition to the six constant temperatures employed, some pans were held at 15° or 20° C. for 16 hr., and at 30° or 35° C. for 8 hr. of each 24-hr. period preceding emergence. This variation in environment also failed to produce infection.

Seed of three sorghum selections was then inoculated with spores of *Sphacelotheca holci* and planted in soil kept at 20°, 25°, or 30° C., until after emergence. The percentages of infection at 20°, 25°, and 30° C., respectively, in the three selections were: Kafir × feterita (K.B. 2510), 14.0, 15.2, and 14.6; kafir × feterita (K.B. 2686), 5.6, 2.4, and 4.9; Red Amber × feterita (K.B. 2501), 14.0, 10.4, and 23.4. The averages of the percentages of infection in the three varieties at 20°, 25°, and 30° C. were 11.5,

[2] The writers appreciate the aid of J. C. Stephens, Chillicothe, Texas, the late C. J. King, Sacaton, Arizona, and others, who sent in this material.

9.6, and 14.7, respectively. No significant difference due to temperature was apparent.

Effect of Temperature on Smut Development in Growing Plants

Some observations seem to indicate that hot weather may not be conducive to smut infection in Johnson grass, since smutted panicles are generally more prevalent in the spring and fall than in midsummer. Smut-infected plants transplanted from the field to pots in the greenhouse in the spring produced only smutted panicles. The culms that were cut back in early July produced healthy panicles in late August. Greenhouse temperatures during this period ranged from 25° to 40° C.

Other smut-infected plants transplanted to an outdoor plot of very poor soil in June 1945 and cut back produced all smutted panicles by July 31. These culms were all cut back again, and after 2 months of hot weather produced mostly smut-free panicles. The culms were cut back again in early October and produced a nearly smut-free crop the following summer.

A smut-infected Johnson grass plant transplanted to a greenhouse bed in March 1946 spread rapidly and sent up many stems, all of which produced infected heads. These were cut back and in mid-October 200 smut-infected heads and only four normal heads were counted. In this case, hot weather had not suppressed smut development.

Smut-infected plants transplanted to an outdoor bed in June 1946 produced an abundance of heads by mid-August and all except one were smutted. Again high temperatures did not prevent smut development. It is possible that factors other than temperature play a part in the seasonal variation of smut in Johnson grass.

Method of Infection and Spread

It seemed advisable to determine whether the smut spreads through the rhizomes and other parts of the Johnson-grass plant. Smut-free plants transplanted to greenhouse pots after being cut back were inoculated hypodermically with a water suspension of chlamydospores of *Sphacelotheca holci* when new shoots appeared. All of the inoculated shoots produced smut-infected heads. Healthy heads developed from the shoots that had not been inoculated. All culms were cut back periodically after producing heads and, after 12 months, 137 smutted heads and 24 smut-free heads were counted in the final crop. It was apparent that the smut mycelium had become established in the crown of the plants, from which place it invaded the new shoots.

Two smut-infected clones transferred to a greenhouse bench, where the spread of the rhizomes was not restricted appreciably, sent up shoots at distances of 3 to 30 in. from the original base, and produced smutted heads, with a few exceptions. Pieces of infected rhizomes from a smutted plant were planted in pots, and later produced culms with smut-infected heads.

These results leave no doubt that the smut mycelium travels throughout the crown, stems, and rhizomes of the plant.

Two uninoculated control plants that produced healthy heads at first were cut back periodically. After the second cutting several of the heads were smutted. Finally, after being cut back eight times, these two plants produced 30 smutted heads and only one that was smut-free. The source of this infection was not apparent.

In 1944, Fisher (1) produced stem smut (*Ustilago speyazzinii* Hirsh.) in crested wheatgrass, *Agropyron cristatum* (L.) Beauv., and slender wheatgrass, *A. trachycaulum* (Link) Malte, by spraying the cut ends of the stubble with a spore suspension. This suggested that infection by Johnson-grass smut might be induced in that manner. Smut-free plants were cut back, and the cut ends of the stubble were sprayed with a water suspension of viable spores of *S. holci* collected from Johnson grass, or were dusted with dry spores (results in table 1).

TABLE 1.—*Total and smutted heads in Johnson grass in the greenhouse following inoculation of the stubble with spores of Sphacelotheca holci*

Row No.	Form of inoculum applied to stubble	Heads		
		Total	Smutted	
		Number	Number	Percentage
1	Spore suspension	50	40	80.0
2	Do	40	30	75.0
3	Dry spores	40	25	62.5
4	Do	55	35	63.6
5	Do	35	20	57.1

It was shown that *Sphacelotheca holci* from Johnson grass also can invade the cut ends of sorghum plants and thus bring about infection. Several smut-free sorghum plants, growing near mature infected Johnson grass, had been cut back; when new heads developed, they were found to be smut-infected, and the culms were severely stunted. The smut was identified as *S. holci*. The new growth that developed after the culms were again cut back was stunted and smutted more severely than the previous growth had been. After a third cutting, only a few weak smutted shoots developed, and the plants died.

This type of infection was duplicated in another sorghum plant that normally grew to a height of about 16 ft. The stubble of one stalk apparently became infected, for the new growth attained a height of only 6 ft. and produced a smutted head, whereas the rest of the culms reached the usual height of 16 ft. and produced healthy heads. All of the stalks were then cut back and spores of *S. holci* were applied to the stubble. The next stalks produced were severely stunted and all had smutted heads, along with a number of smutted and deformed axillary branches. Another cutting was followed by a smutted and more severely stunted crop that

died a few weeks after ripening. Red kafir, similarly cut back and inoculated, was not affected.

Other healthy plants of a susceptible variety of sorghum growing among smutted plants also became smutted after they were cut back.

From these results, it may be concluded that the mowing of Johnson grass that is partly infected with smut is likely to insure a heavier infection in the following crop, because of the spores falling upon and causing invasion through the stubble. It is also reasonable to suppose that, in a field infested with or bordered by smut-infected Johnson grass, a second-growth crop of a susceptible variety of sorghum could become infected with *S. holci* in this manner.

Varietal Susceptibility to Sphacelotheca holci

In experiments in 1944, *Sphacelotheca holci* infected feterita × kafir (K.B. 8917), kafir × feterita (K.B. 2510 and 2686), and Red Amber × feterita (K.B. 2501). It did not infect Pierce kaferita. Johnston et al. (4) in 1936 failed to get infection in K.B. 2686 and also in Pierce kaferita.

TABLE 2.—*Infection in 14 varieties or selections of sorghum grown from seed, inoculated with spores of two physiologic races of loose kernel smut (Sphacelotheca cruenta) from sorghum, and S. holci from Johnson grass, and planted in 30-ft. rows on the South Farm, Plant Industry Station, Beltsville, Maryland, May 22, 1945*

Variety or selection	Accession number[1]	Percentage of plants infected with		
		S. cruenta		*S. holci*
		Race 1	Race 2	
Early White milo	C.I. 3026	45.5	0.0	0.0
Pierce kaferita	K.B. 2547	17.1	3.8	0.0
Feterita	C.I. 51989	9.1	0.0	0.0
Kafir × feterita	K.B. 2686	21.8	8.6	0.0
Red Amber × feterita	K.B. 2501	7.7	16.7	5.0
Do	K.B. 2570	0.0	42.6	5.0
Do	K.B. 2573	0.0	4.9	0.0
Do	K.B. 2562	0.0	15.4	0.0
White Yolo	K.B. 2525	25.0	0.0	0.0
Kafir × feterita	K.B. 2510	20.0	5.2	0.0
Reed kafir	C.I. 628	56.1	28.9	0.0
Sharon kafir		58.5	23.8	0.0
Red kafir	C.I. 957	75.0	44.6	0.0
Dwarf Yellow milo	C.I. 332	0.0	0.0	0.0

[1] C.I. and K.B. are accession numbers of the Division of Cereal Crops and Diseases and the Botany Department, Kansas State College, respectively.

In 1945, spores of *Sphacelotheca holci* and of races 1 and 2 of *S. cruenta* were applied to seed of 14 sorghum varieties or crosses. *S. holci* infected only Red Amber × feterita (K.B. 2501 and K.B. 2570) (Table 2). The two races of *S. cruenta* produced infection in 13 selections.

In an additional pathogenicity test, seed of 183 sorghum varieties, selections, or crosses was inoculated with viable spores of *S. holci* and planted in 30-ft. rows. A trace of infection was found only in shallu and Bonita.

Again in 1946, seed of 197 selections or varieties of sorghum was similarly inoculated with *S. holci* and planted, but only 21 of these produced any visible evidence of infection (Table 3). These negative results are not considered highly significant, however, because the stands of many selections were poor, the number of plants per row ranging from only 8 to 90. It is possible that some plants in other varieties became infected but were so severely stunted that they escaped detection when data were taken.

TABLE 3.—*Varieties or selections of sorghum that showed susceptibility to Sphacelotheca holci when grown from inoculated seed at Plant Industry Station, Beltsville, Maryland, in 1946*

Variety or selection	Accession number[1]	Plants		
		Number	Smutted	
			Number	Per cent
Spur feterita	C.I. 623	24	2	8.5
Common feterita	C.I. 182	20	1	5.0
Early hegari	S.A. 281	50	14	28.0
Hegari	F.P.I. 34911	50	14	28.0
Arizona hegari	S.A. 278	25	2	8.0
Wonder	F.C. 8986	50	1	2.0
Ajax	F.C. 6620	36	3	8.5
Imperial kafir	S.A. 360	50	8	16.0
Grohoma	F.C. 16188	80	2	2.5
Fredoka	S.A. 279	80	3	3.8
Shallu	———	56	7	12.5
Sumac	P.I. 35038	80	1	1.3
Logera	———	24	8	33.3
Feterita × kafir	F.C. 8917	60	4	6.7
Kafir × feterita	K.B. 2510	65	4	6.2
Do	K.B. 2686	70	11	15.7
Do	H.C. 2423	50	25	50.0
Red Amber × feterita	K.B. 2501	66	8	12.1
Do	K.B. 2562	80	11	13.8
Do	K.B. 2570	70	4	5.7
Do	K.B. 2573	55	3	5.5
Johnson grass	F.C. 22855	18	6	33.3

[1] S.A., P.I., F.C., and H.C. are accession numbers of the Texas Agricultural Experiment Station, the Division of Plant Exploration and Introduction, the Division of Forage Crops and Diseases, and the Hays (Kansas) Branch Experiment Station, respectively.

Some of the infected plants in the varieties listed in table 2 were only 4 to 5 in. high and consisted of one slender stem bearing a small imperfect head, whereas the uninfected plants were 6 to 10 ft. high.

With the exception of shallu, Sumac sorgo, and the three varieties of hegari, all of the susceptible sorghums listed in table 3 are of known or probable feterita origin. None of the kafir or milo varieties was infected.

In 1946, separate lots of seed of Johnson grass, three selections of Sudan grass, and 16 varieties or crosses of sorghum were inoculated with spores of *Sphacelotheca holci* and the two races of *S. cruenta*. The seed was planted in 30-ft. rows on June 4. Data on infection are in table 4.

Five of the selections were infected by all of the smuts; five were in-

fected by both races of *Sphacelotheca cruenta* but not by *S. holci;* three were infected by *S. holci* and race 2 of *S. cruenta;* Johnson grass was infected only by *S. holci.* The three selections of Sudan grass showed no infection by *S. holci.* However, Rodenhiser (5) in 1936 obtained an average of 16.9 per cent infection in Sudan grass (F.C. 22265). This difference in results may be attributed to differences in conditions for infection, or in the susceptibility of different strains of Sudan grass. The existence of physiologic races of *S. holci* also is a possibility.

TABLE 4.—*Infection in varieties or selections of sorghum, Sudan grass, and Johnson grass grown from seed inoculated with spores of two races of loose kernel smut (Sphacelotheca cruenta) from sorghum, and loose kernel smut (S. holci) from Johnson grass, and planted in 30-ft. rows at the Plant Industry Station, Beltsville, Maryland, in 1946*

Variety or selection	Accession number[1]	Percentage of plants infected with		
		S. cruenta		*S. holci*
		Race 1	Race 2	
Early White milo	C.I. 3026	40.9	12.7	0.0
Pierce kaferita	K.B. 2547	5.9	2.7	0.0
Feterita × kafir	F.C. 8917	10.8	1.3	6.7
Do	K.B. 2510	18.8	6.3	6.2
Do	K.B. 2686	23.6	15.9	15.7
Do	H.C. 2423	40.0	22.2	50.0
Feterita	C.I. 51989	2.2	5.1	0.0
Red Amber × feterita	K.B. 2501	6.0	22.2	12.1
Do	K.B. 2562	0.0	11.9	13.8
Do	K.B. 2570	0.0	1.8	5.7
Do	K.B. 2573	0.0	1.7	5.5
White Yolo	K.B. 2525	0.0	0.0	0.0
Red kafir	C.I. 957	66.7	64.1	0.0
Reed kafir	C.I. 628	61.4	63.2	0.0
Sharon kafir		53.3	50.0	0.0
Dwarf Yellow milo	C.I. 332	0.0	0.0	0.0
Sudan grass	F.C. 22827	15.0	6.0	0.0
Sudan grass, Tift	F.C. 22919	25.0	10.0	0.0
Sudan grass, Sweet	F.C. 31868	20.0	9.0	0.0
Johnson grass	F.C. 22855	0.0	0.0	33.3

[1] C.I. and K.B. are accession numbers of the Division of Cereal Crops and Diseases and the Botany Department, Kansas State College, respectively. F.C. and H.C. are accession numbers of the Division of Forage Crops and Diseases and the Hays (Kansas) Branch Experiment Station, respectively.

It is of interest that all of the sorghum selections infected by *S. holci* in this experiment are of feterita origin, and that the kafirs, which are highly susceptible to all forms of both *S. cruenta* and *S. sorghi,* appear to be immune from *S. holci.*

In another experiment, *Sphacelotheca sorghi* was included along with *S. holci* and a mixture of the two races of *S. cruenta* to test further the susceptibility of Johnson grass to the common sorghum smuts, and the susceptibility of Blackhull kafir to *S. holci.* Kafir × feterita (H.C. 2423), which is susceptible to all of these smuts, was included as a check.

Each of the smuts was applied to a separate portion of the above seed

lots. The seed was planted in 20-ft. rows, replicated three times. Plants from uninoculated seed of each lot planted as a check remained smut-free. Data on infection are in table 5. Johnson grass proved to be slightly susceptible to *S. cruenta* but not to *S. sorghi*, while kafir maintained its immunity from *S. holci*.

TABLE 5.—*Relative infection by Sphacelotheca holci, S. cruenta, and S. sorghi in Johnson grass, kafir × feterita (H.C. 2423), and Blackhull kafir when grown from surface-sterilized seed artificially inoculated with spores of these smuts and planted in replicated 20-ft. rows*

Host	Replication	Total and smutted plants from seed inoculated with					
		S. holci		*S. cruenta*		*S. sorghi*	
		Total	Smutted	Total	Smutted	Total	Smutted
Johnson grass	1	45	15	30	0	50	0
	2	10	5	25	0	40	0
	3	10	4	18	2	10	0
	4	.....	.....	52	3	.....	.....
	Total	65	24	125	5[1]	100	0
	Pct.	.....	37	.....	4	.....	0
Kafir × feterita (H.C. 2423)	1	40	3	76	6	50	4
	2	60	10	43	9	54	5
	3	37	3	53	14	67	4
	Total	137	16	172	29	171	13
	Pct.	.....	11.7	.....	16.9	.....	7.6
Blackhull kafir	1	57	0	73	30	55	25
	2	50	0	50	25	57	25
	3	84	0	64	26	61	33
	Total	191	0	187	81	173	83
	Pct.	.....	0	.....	43.3	.....	48

[1] These plants were not stunted so severely as those infected with *S. holci*, and the smutted panicles were different. The periderm was not broken and the smut balls were whole, as generally is the case in the earlier stages of *S. cruenta* but not in *S. holci*. The spores were smaller and less prominently echinulate than those of *S. holci*.

Effect of Sphacelotheca holci on Host Plants

One of the pronounced differences between the symptoms produced in sorghum by covered kernel smut, *Sphacelotheca sorghi*, and those produced by loose kernel smut, *S. cruenta*, is the tendency of the latter to stunt the infected plants, whereas *S. sorghi* causes no visible symptoms until the smutted heads appear. Race 2 of *S. cruenta* in general has been more severe in this stunting effect than has race 1. *S. holci* causes even more stunting than does race 2 of *S. cruenta*. In 1944, measurements of healthy and infected plants showed that race 1 and race 2 of *S. cruenta* reduced the average height of plants 7.5 and 22.4 per cent, respectively, whereas *S. holci* reduced it 67.3 per cent. In 1945, similar measurements of infected and healthy plants of Red Amber × feterita (K.B. 2501) showed the corresponding figures for these three smuts to be 6, 19, and 63 per cent, respectively.

In 1946, races 1 and 2 of *S. cruenta* reduced the average height of plants of four selections of sorghum only 8.2 and 9.7 per cent respectively, while *S. holci* caused an average reduction of nearly 65 per cent (Table 6).

Spore Studies

In addition to its host range, its effect upon susceptible varieties, and its method of spread, *Sphacelotheca holci* differs from *S. cruenta* in the size, color, character, germination, and longevity of its chlamydospores. Johnston et al. (4) reported that the chlamydospores of *S. holci* averaged about 2 μ more in diameter and were darker and more prominently echinulated than those of *S. cruenta*. Its promycelia and sporidia also were somewhat longer. Similar observations were made by the writers, who also noted that over 15 per cent of the germinating spores of *S. holci* produced two promycelia, whereas in *S. cruenta* this phenomenon occurred infrequently.

TABLE 6.—*Reduction in height of sorghum plants infected by Sphacelotheca cruenta (races 1 and 2) and S. holci*

Variety or selection	Accession number	Average height of healthy plants	Reduction in height due to		
			S. cruenta		*S. holci*
			Race 1	Race 2	
		Inches	Per cent	Per cent	Per cent
Feterita	F.C. 8917	76.8	11.6	15.2	68.1
Kafir × feterita	K.B. 2686	50.6	9.9	8.1	60.1
Do	H.C. 2423	46.3	0.0	0.6	63.3
Red Amber × feterita	K.B. 2501	76.4	11.1	14.8	67.9
Average		62.5	8.2	9.7	64.9

One of the most pronounced differences between the spores of these two species is in their relative longevity. Spores of *S. cruenta* stored in the laboratory at room temperature have remained viable for as long as 4 years, but spores of *S. holci*, similarly stored, lost their viability within a few months. When stored at 10° C. they have remained viable for as long as 20 months, but their germination after that period was less vigorous than when they were fresh. However, it is doubtful that their period of viability in the field exceeds 2 or 3 months.

DISCUSSION

Rapid loss of spore viability, infection of plants through stubble, and the spread of mycelium through the rhizomes explain why *Sphacelotheca holci* spreads rapidly in Johnson grass and yet rarely infects susceptible varieties of sorghum growing near by. Johnson-grass plants grown from seed in the current season are rarely smutted because the smut spores fail to survive the period between seed harvest and planting time.

Although sorghum plants are readily infected through the stubble, the season is rarely long enough for a second crop of heads to develop. Sorghum stubble may become infected frequently, but since no heads are produced the smut fungus fails to produce spores.

Most of the grain sorghum now grown consists of varieties with kafir-milo parentage. Neither these varieties, nor their parental types, seem to be susceptible to *Sphacelotheca holci*. Most varieties of sorgo, broomcorn, and Sudan grass also appear to be resistant. The usual growing of a single crop of sorghum in a season, together with the fact that most sorghum varieties are resistant, suggest that Johnson-grass smut is not a serious threat to the sorghum crop under present conditions.

SUMMARY

The smut commonly found infecting Johnson grass apparently is not a physiologic race of *Sphacelotheca cruenta* but is *S. holci* Jackson, originally found on cultivated sorghum in Venezuela. The spores are larger, darker, more prominently echinulate, and shorter-lived than are those of *S. cruenta*. Infection may take place through inoculation of seed or of stubble. The mycelium spreads throughout the crown and through the rhizomes of Johnson grass. A definite relation of temperature to infection was not established.

The host range of *Sphacelotheca holci* in the sorghums is more limited than is that of the loose kernel smut in sorghum, *S. cruenta*. With few exceptions, the sorghums that proved susceptible to *S. holci* are of known or probable feterita origin. The kafirs appear to be immune. Johnson grass is slightly susceptible to *S. cruenta* but appears to be immune from *S. sorghi*.

Smut from Johnson grass causes a more severe stunting in sorghum than does the loose kernel smut from sorghum, and it sometimes kills the infected plants.

The fact that the spores of *Sphacelotheca holci* are relatively short-lived prevents this smut from being a menace to sorghum culture.

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INFLUENCE OF TEMPERATURE ON INCUBATION AND TRANSMISSION OF THE WOUND-TUMOR VIRUS[1]

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(Accepted for publication May 12, 1950)

Wound-tumor disease is caused by the virus *Aureogenus magnivena* Black (3).[3] Infected plants of many different species in numerous plant families usually have irregularly enlarged leaf veins and tumors on the roots; in sweet clover (*Melilotus alba* Desr.), tumors also occur on the stems. Because wounds start tumors of potentially unlimited growth in certain plants systemically invaded by the virus, it has been named wound-tumor virus (4). In spite of many efforts to transfer the virus by rubbing or injecting juice from diseased plants into healthy ones (20), it has never been transmitted mechanically from plant to plant. It is readily transmitted by grafting and, after an incubation period, by the three leafhoppers *Agallia constricta* van Duzee, *Agallia quadripunctata* (Provancher), and *Agalliopsis novella* (Say). The principal aim was to find the influence of temperature on transmission and on the minimum incubation period of the wound-tumor virus in the insect *A. constricta* and in the plant.

Several other interesting problems that arose were investigated: incubation periods were studied in insects injected with different dilutions of virus; the minimum incubation period was compared in two species of vectors and in punctured and unpunctured insects; observations were made on virus-free cuttings from infected plants (19); the incubation period and susceptibility to infection were studied in plants of various ages.

LITERATURE

The influence of environmental factors on virus incubation periods has often been observed. In 1924 Severin (23) reported that in greenhouse experiments the length of incubation periods of the virus of curly top of sugar beets (*Ruga verrucosans* Carsner & Bennett) in the leafhopper *Eutettix tenellus* (Baker) depended on the temperature at which the insect was held. In 1928 Storey (24) found differences in incubation periods

[1] The investigation was carried out during the tenure of a Brooklyn Botanic Garden Fellowship (1947–1948) and a Fellowship in Cancer Research from the American Cancer Society (1948–1949), recommended by the Committee on Growth of the National Research Council.

The work herein reported was part of a thesis presented in May 1949 in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Faculty of Pure Science, Columbia University.

[2] The author is indebted to Dr. L. M. Black, Brooklyn Botanic Garden, for encouragement and support during the progress of the research and preparation of the manuscript.

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[3] Throughout the paper Holmes' binomial system of virus nomenclature has been followed (11).

of maize streak virus, *Fractilinea maidis* (Holmes) McKinney, in *Cicadulina mbila* Naude, held at four different controlled temperatures. Storey's experiments were made with a limited number of individuals, only five insects having been tested at each temperature. Nevertheless, Storey's work was important as the first attempt to study the influence of different controlled temperatures on the incubation period of a plant virus in its insect vector. Storey did not have the facilities to carry out similar experiments on the incubation period in the plant. The incubation of a plant virus both in the plant and in the vector under constant conditions of controlled light and temperature has not previously been studied. A brief report on the results of early experiments with one of the vectors was published in 1949 (18).

MATERIALS AND METHODS

Agallia constricta was used in all experiments unless otherwise stated. The insects were obtained from stock cultures of nonviruliferous leafhoppers raised in the insectary at the Brooklyn Botanic Garden. The stock colonies were routinely checked for noninfectivity; besides, nonviruliferous insects were included as controls in experiments, and none of them ever transferred the wound-tumor virus during the course of the work.

Frequent fumigations, usually once a week, with tetraethyl-pyrophosphate bombs reduced the possibility of leafhopper contamination of the greenhouse and provided control against red spider, thrips, white fly, and aphid pests. In order to avoid eggs being laid in the test plants, impregnated females were not used in the experiments. As soon as nymphs being tested in colonies changed into adults, females and males were separated and colonies were split into three groups and tested on three plants instead of a single one. Whenever one, two, or all three of the groups transmitted, the result was indicated in the tables by a single sign as belonging to a single colony. A record was always kept of the number of insects in each colony at each transfer. This was desirable because loss of insects by death reduced the size of the colonies while the experiments were in progress.

Crimson clover plants (*Trifolium incarnatum*) were used both as source and test plants because of their high susceptibility to the virus and uniform response to infection. The test plants were grown from commercial seed, germinated in a mixture of peat and sand, and transplanted to 2½-in., 4-in., or 6-in. pots. Insect cages were made out of transparent celluloid. The tops of the cages were also made of celluloid instead of the usual cheesecloth; this was necessary because of the low light intensity in the experimental chambers described below. Cages were provided with side openings covered with cheesecloth. The ventilation inside the cages apparently was adequate since no condensation of water vapor occurred. The insect catcher devised by Kunkel (13) was employed in insect transfers. Nonviruliferous insects from stock cultures were allowed to feed on source plants for varying periods of time and were then transferred to successive

healthy crimson clover plants at 24- or 48-hr. intervals, usually for 40 or more days. After inoculation, exposed test plants were removed from the cages and chambers and held under observation for 60 days in the greenhouse. The number of insects tested exceeded 1300, and more than 6000 plants were used in the experiments.

After preliminary tests in the greenhouse during the summer of 1947, chambers were built[4] and insects under test were kept at constant temperatures under artificial light. Alternating periods of 16 hr. of light and 8 hr. of darkness were used in all experiments. Each chamber (Fig. 1), measuring $80 \times 110 \times 120$ cm., was lighted by 28 fluorescent tubes providing light at an intensity of 560 meter candles. The tubes were installed outside each chamber over a plastic transparent ceiling of cellulose acetate.

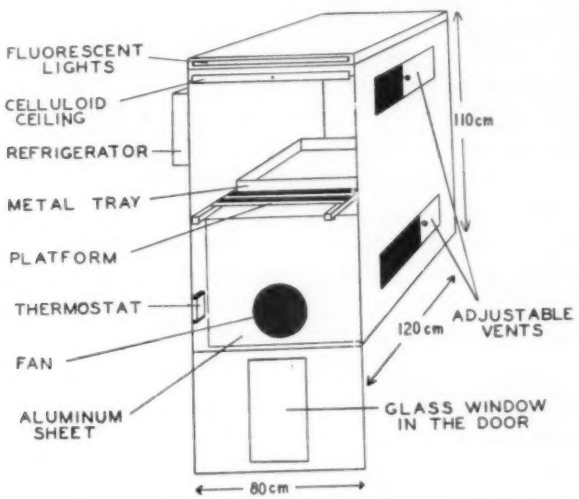

FIG. 1. Diagram of a chamber (upper door removed).

High-pressure axial flow fans provided air circulation. Two of the chambers had refrigerating units and in one chamber a heating coil was placed in front of the fan. Temperature controllers of the gas bulb type were used; temperature fluctuations and variations in different parts of a chamber were of the order of $\pm 0.5°$ C. Although air temperatures were practically constant for the duration of an experiment, differences of the order of $1°$ to $2°$ greater than desired were found near the top inside insect cages during the light periods. In darkness these differences did not occur. The soil temperature of potted plants was always about $2°$ lower than that of the air in the chamber. Insects feeding close to the base of plants or sitting on the ground were therefore in a somewhat different environment from that of insects feeding higher up. Adjustable vents placed in the separating walls allowed an interchange of air between the chambers; three holes, 1 in. in diameter, at one end of the series of three chambers

[4] The author wishes to acknowledge the assistance rendered by Dr. M. K. Brakke in connection with the building and operating of the temperature control chambers.

permitted outside air to enter the chamber; three holes in the other end chamber allowed the air to flow out.

DEFINITION OF TERMS

The following terms are used in describing the relationship between the plant, vector, and virus.

Infection feeding: The time that a previously noninfective vector is fed on a virus source (26).

Test feeding: The time that a vector is fed upon a healthy recipient test plant (26).

Incubation period in the insect: The time between acquisition and transmission of virus by the vector.

Infective insects: Vectors that have passed through the incubation period and are able to transmit the virus.

Viruliferous insects: Vectors bearing virus, whether they have passed through the incubation period or not.

Source plant: A plant infected with the virus and employed as a source of virus for insects undergoing an infection feeding.

Test plant: A healthy susceptible plant employed to determine whether any insect or colony of insects is transmitting.

Incubation period in the plant: The time between the first day of infective test feeding and the first day symptoms appear.

EXPERIMENTS AND RESULTS

In the incubation period experiments, insects were kept from the first until the last day at a given temperature, first for the infection feeding and then for the test feedings.

The first experiment on the incubation period of the virus in *Agallia constricta* was performed in the greenhouse. Sixty nonviruliferous nymphs and sixty nonviruliferous adult males were allowed to feed on a source plant for 9 days. Then the insects were divided at random in colonies of 10 nymphs or 10 males and transferred every second day to healthy crimson clover plants (Table 1). The 2-day transfers were used in most experiments instead of shorter intervals because it was found that the vectors do not transmit the virus regularly every day. Each of the 2-day test feeding periods is indicated in the table by the number of the first day only. For example, 12 represents the 12th and 13th days the insects were fed on test plants; 14, the 14th and 15th days, etc. Twenty insects in two colonies were included as controls; they were placed on a healthy crimson clover plant for the first 9 days and later transferred in the same way as all other colonies. The control insects did not infect any plants.

Both nymphs and adults picked up virus and became infective. Although the shortest incubation period was 23 days for both nymphs and adults, some colonies required as much as 39 days before they became infective. Transmission by infective colonies did not occur regularly; on

some days almost all plants were infected, whereas on others only one or two succumbed. These results suggested the possible influence of external factors on transmission and led to the experiments with controlled temperature and light.

It was important for the calculation of incubation periods to find out whether colonies of 10 insects were able to acquire the virus during an infection feeding of a single day. In only one experiment, at 27.5° C., did all five tested colonies pick up the virus during 1 day of feeding on the source plant. Data from several other experiments proved that at other less favorable temperatures a test feeding of 1 day was insufficient for most insects to acquire the virus.[5] Therefore, almost all experiments on

TABLE 1.—*Incubation period of wound-tumor virus in Agallia constricta in the greenhouse. Colonies of 10 insects (No. 1–No. 6 nymphs, No. 7–No. 11 adults)*

Colony	Infection feeding	Transmission record[a] Days (from start of experiment)																
		10	12	14	16	18	20	22	24	26	28	30	32	34	36	38	40	42
1	9	−	−	−	−	−	−	−	+	+	+	−	−	−	−	−	+	−
2	days	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+
3		−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+
4		−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+
5		−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−
6		−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+
7		−	−	−	−	−	−	−	+	−	−	+	−	+	+	+	+	−
8		−	−	−	−	−	−	−	−	−	+	−	+	−	+	+	+	+
9		−	−	−	−	−	−	−	−	−	−	+	−	−	−	+	+	+
10		−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−
11		−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+
Control 1		−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Control 2		−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−

[a] + indicates plant diseased; − indicates plant healthy.

incubation periods in the insect were performed with infection feedings of from 7 to 10 days' duration, periods always less than the incubation period discovered. For practical reasons the incubation period was reckoned as the time elapsing from the beginning of the infection feeding to the beginning of the 2-day test feeding period in which the insect or colony first transmitted. Although a considerable number of insects acquired the virus later than on the first day of infection feeding, the feedings of several days were deemed as good as or superior to those of one day, since the minimum period at a particular temperature was sought. The method of calculation was based on the assumption that infection feeding of more than 1 day would in no case lengthen the incubation period for a colony of insects, and in some cases might shorten it. Longer infection feeding proved also more reliable in providing good transmission at various temperatures.

[5] Dr. L. O. Kunkel, in a personal communication, indicated that in insect transmission studies on aster yellows virus only about 35 per cent of the vectors used acquired the virus during the first day of infection feeding; not until the 14th day did all of the insects become viruliferous.

Incubation Period at Different Temperatures

It is possible that source plants vary in virus content at different times of the year and thus have an effect upon the acquisition of virus by colonies of vectors. The vitality of insects at the start of several experiments also differed. This was attributable, in part at least, to the relatively poor growth of the host plants during certain months of the year. Hence, comparisons of different temperatures were considered more reliable if they were made in the same experiment. It was not feasible to carry out all experiments simultaneously because of the large number of insects involved. The fact that a temperature near the optimum was included in each experiment makes rough comparisons possible among all temperatures tested.

The influence of different temperatures was tested at 16°, 20°, 25°, 26°, 27.5°, 30°, 32.5°, and 37° C. (Table 2). The plants employed at 37° were prewarmed before the test feeding by keeping them at 37° for 1 or 2 days. In a similar manner, plants used at 16° were cooled before using.

It was found that temperature had a pronounced influence on the incubation period and also on transmission. At 16° and at 20° the period was at least 30 days and only a few colonies transmitted the virus. The minimum incubation period, summarized as 13 to 15 days, was found at temperatures ranging from 25° to 32.5° C. No plants became infected at 37° C. In two experiments the time required for metamorphosis from nymphs to adults was indicated. At 16° this first occurred after 78 days (not shown in the table), at 26° after 25 days, and at 37° after only 21 days. Metamorphosis from nymph to adult had no influence on the incubation period.

The minimum incubation period of the wound-tumor virus in the insect was 13 to 15 days, whether the infection feeding lasted one or several days. At present there is only one other plant virus known to have an incubation period of a similar length. Kunkel (17) found that the virus of corn stunt had an incubation period of 14 days and he stated that it is the longest incubation period for any plant virus that has been adequately studied.

It was found in a preliminary test that insects were able to acquire the virus at 20° C., but the transmission at that temperature was poor. When it was found that a 30-day incubation period occurred at 20°, an attempt was made to ascertain whether this long period was real or poor transmission accounted for the results. This was tested in the following way: 300 nonviruliferous insects were caged for a 7-day infection feeding at 20° C. on a source plant. Next they were kept, still at 20°, on alfalfa plants (*Medicago sativa* L.) of the variety Grimm, immune from wound-tumor. Every 48 hr. ten insects were removed from the 20° chamber and caged on crimson clover plants at 27.5° for two successive periods of 2-day

test feedings. It was found (Table 3) that insects kept at 20° did not infect test plants before the 28th day, although they were tested at a temperature suitable for transmission. Since the two transmissions on the

TABLE 2.—*Incubation period of wound-tumor virus in Agallia constricta at various temperatures. Colonies of 10 insects*

Temperature and colony number	Transmission record[a] Days (from start of experiment) 14	16	18	20	22	24	26	28	30	31	33	35	37	39	41	43	45	47
Experiment A, 7 days' infection feeding																		
20° C.																		
1	–	–	–	–	–	–	–	–	–	–	–	–	+	+	+	–/	–	–
2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	+/	+
3	–	–	–	–	–	–	–	–	–	–	–	+	+	–	–	–	–/	–
4	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–/	+
5	–	–	–	–	–	–	–	–	–	+	–	+	–	–	+	+	–/	+
Control	–	–	–	–	–	–	–	–	–	–	–	–	–/	–	–	–	–	–
25° C.																		
6	–	–	+	–	+	+	+	+	–/	+	–	+						
7	–	–	–	+	+	+	+	+	–	+/	–	–						
8	–	+	–	+	+	–	+	+	–/	+	–	+						
9	–	–	–	–	–	–	+	+/	+	–	+	+						
10	–	–	–	–	–	–	+	+/	+	+	+	–						
Control	–	–	–	–	–	–	–	–/	–	–	–	–						
30° C.																		
11	–	–	–	–	–	+/	+	+	–	+	+	+	–	+	+	+	–	+
12	–	–	–	–	–	–/	–	–	–	–	–	–	–	–	–	–	–	–
13	–	+	–	+	–	–	+/	+	+	–	+	+	+	+	+	+	–	–
14	–	–	+	+	+	+	+/	+	+	+	+	+	+	+	+	+	+	+
15	–	–	–	–	–	+	+/	+	+	+	+	+	+	+	–	–	+	–
Control	–	–	–	–	–	–/	–	–	–	–	–	–	–	–	–	–	–	–

	Days (from start of experiment) 14	16	18	20	22	24	26	28	30	32	34	36	38	40	42
Experiment C, 7 days' infection feeding															
27.5° C.															
1	+	–	+	–	+	+	–	+	+	+	+	+	+	+	+
2	–	–	+	–	–	+	+	+	+	+	+	+	+	+	+
3	–	–	–	+	+	+	+	+	+	+	+	+	+	+	–
4	–	–	+	–	–	+	–	+	–	–	+	+	–	–	–
5	–	–	+	–	–	–	–	+	–	+	–	–	+	–	+
Control	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
32.5° C.															
6	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+
7	–	+	+	–	+	+	–	–	+	+	–	–	–	–	–
8	–	–	+	+	–	–	+	+	+	–	–	+	+	+	+
9	–	–	–	+	+	–	+	+	+	–	+	+	–	+	–
Control	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

[a] – indicates plant healthy; + indicates plant diseased. All plants were healthy on the 8th, 10th, and 12th days.

Diagonal line indicates that first insect changed to adult.

22nd and 26th day occurred in colonies previously tested for 2 days at 27.5° with negative results, these data have no bearing on the interpretation of the results. The tests indicated that the long incubation period at 20° was real and not due to poor transmission.

TABLE 2.—Continued.

Temperature and colony number	Transmission record[b] Days (from start of experiment)																								
	11	13	15	17	19	21	23	25	27	29	31	33	35	37	39	41	43	45	47	49	51	53	55	57	59	61
Experiment B, 10 days' infection feeding																										
16° C.																										
1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
4	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	+	–	–	–	–	+	–	–	+	–
5	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–
Control	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
26° C.																										
6	–	–	–	–	+	+	+	+	+	+	+	+	+	+	+/	–	+	+	+	+*						
7	–	–	–	–	–	+	+	+	+	+	+	+	+/	+	+	+	+	+	+	+	–	–	+	+	+	+
8	–	–	+	+	–	+	+	+	+	+	+	+	+/	–	+	+	+	+	+	+	+	+	+	–	–	+*
9	–	–	–	+	–	+	+	+	+	+	+/	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+
10	–	–	–	+	–	+	+	+/	+	+	+	+	+	+	+	+	+	+	+*							
Control	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
37° C.																										
11	–	–	–	–	–	–	–	–	–/	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
12	–	–	–	–	–	–	–	–	–	–	–	–	–/	–	–	–	–	–*								
13	–	–	–	–	–	–/	–	–	–	–	–	–	–	–	–	–*										
14	–	–	–	–	–	–	–	–/	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–*	
15	–	–	–	–	–	–	–/	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Control	–	–	–	–	–	–	–/	–	–	–	–	–	–	–	–	–	–	–	–	–	–*					

[b] – indicates plant healthy; + indicates plant diseased.
Diagonal line indicates that first insect changed to adult. At 16° C. this change occurred first in colony 5 on the 78th day (not indicated in table).
Asterisk indicates death of last insect in colony.

Transmission at Different Temperatures

To investigate the influence of temperature on transmission apart from incubation, colonies of insects were used which had completed their incubation periods (Table 4, A and B). The transmission at 16° and at 20° was poor; at 26° and at 30° it was much better. At 35° transmission during the first week was fair but later it became very poor. After the first 11 days of test feeding the same colonies of insects (Table 4, B) were transferred from 16° and 35° into the 26° chamber. The insects that came from 16° now transmitted as well as insects kept at 26° all the time, but insects from 35° transmitted very poorly and did not regain their previous infectivity during the time they were tested. It might be that a longer test feeding would show a reactivation of the virus similar to that found by Kunkel (14, 15) for the aster-yellows virus subjected to certain heat treatments.

TABLE 3.—*Incubation period of wound-tumor virus in Agallia constricta at 20° C. Colonies of 10 insects, tested at 27.5° C.*

Colony	Infection feeding	Days (from start of experiment) 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44
1	7 days at 20° C.	− −
2		− −
3		− −
4		− −
5		− −
6		− −
7		− + Test feeding at 27.5° C.[a]
8		− −
9	Feeding on immune plants at 20° C.	− +
10		− −
11		+ +
12		+ +
13		− +
14		+ +
15		− +
16		+ +
17		+ +
18		+ +

[a] − indicates plant healthy; + indicates plant diseased.

Several experiments on transmission were performed with single insects. When 10 insects were tested individually at 25° C. (Table 5, A), some were found infective, whereas others did not transmit the virus during 9 days of test feeding or did so only on a few days. Such failures constituted the chief reason for using colonies of 10 insects rather than single insects in the experiments on incubation.

In another experiment (Table 5, B), the infection feeding at 27.5° C. lasted 36 days. Then the insects were divided into groups of 15 insects and tested individually at different temperatures. At 20° transmission was poor; at 27.5° it was much better. At 32.5° the possibilities for transmission were considerably less due to high mortality.

In this experiment it was demonstrated also that the insects were able to infect healthy plants immediately after leaving infected ones, provided the incubation period had been completed. Discussing various interpretations of the incubation period, Bawden (1) states: "[The latent period] seems to start from the time the vectors leave the infected plant rather

TABLE 4.—*Transmission of wound-tumor virus by colonies of infective Agallia constricta at different temperatures*

Colony	Infection feeding	Subsequent feeding on immune plants	Temperature during test feeding	Transmission record[a] Days (from start of experiment) 30	32	34	36	38	40		42	44	46
			Experiment A. Colonies of 5 insects										
1	7 days	23 days	20° C.	−	+	−	−	−	+				
2	at	at		−	−	−	−	−	−				
3	25° C.	25° C.		−	−	−	−	−	+				
4				+	−	−	−	−	−				
5				−	+	−	−	−	+				
Control				−	−	−	−	−	−				
6	7 days	23 days	30° C.	+	+	+	+	+	+				
7	at	at		+	+	+	+	−	+				
8	25° C.	25° C.		+	+	+	+	+	+				
9				+	−	+	+	+	+				
10				+	+	+	+	+	−				
11				−	+	−	+	−	−				
Control				−	−	−	−	−	−				
			Experiment B. Colonies of 10 insects										
1	7 days	23 days	16° C.	−	−	−	−	−	−		+	+	+
2	at	at		−	−	−	−	+	−		+	+	a
3	26° C.	26° C.		−	+	+	+	−	−	26° C.	+	+	+
4				−	−	+	+	−	+		+	+	+
5				−	−	−	−	+	+		+	+	+
Control				−	−	−	−	−	−		−	−	−
6	7 days	23 days	26° C.	−	+	+	+	+	+				
7	at	at		−	+	+	+	+	+				
8	26° C.	26° C.		−	+	+	+	+	+				
9				−	b	+	+	b	+				
10				−	+	+	+	+	+				
Control				−	−	−	−	−	−				
11	7 days	23 days	35° C.	+	+	−	+	−	−		−	−	+
12	at	at		+	+	+	−	−	−		−	−	−
13	26° C.	26° C.		−	−	+	−	−	−	26° C.	−	+	−
14				+	+	+	+	+	+		+	−	−
15				+	+	−	+	−	−		−	−	−
Control				−	−	−	−	−	−		−	−	−

[a] − indicates plant healthy; + indicates plant diseased.
[b] Plant lost before symptoms could develop.

than from the start of feeding on it. If the latent period were merely the time taken for the virus to reach an infective concentration, there seems no reason why the whole of the period should not be passed on the diseased plants and why adult insects raised on diseased plants should not infect healthy plants immediately." It was pointed out by Kunkel (16) that there is no evidence anywhere in plant virus literature to support

this statement. In fact, for all standard inoculations with wound-tumor virus, insects were kept 4 to 5 weeks on a source plant and then immediately used on test plants.

TABLE 5.—*Transmission of wound-tumor virus by single infective insects at different temperatures*

Insect No.	History of insects prior to test feeding	Temperature during test feeding	Transmission record[a] Days (from start of experiment) 37 38 39 40 41 42 43 44 45 46 47 48
		Experiment A	
1	7 days' infection	25° C.	+*
2	feeding followed		− − − − − − − −*
3	by 31 days on		− − − − − − + + +
4	immune plants		− − − − −*
5	at 25° C.		− +*
6			− − + + + +*
7			− + + + + + − +
8			− −*
9			+ + − − + +*
10			− − − − − − − − −
		Experiment B	
1	36 days' infection	20° C.	− + − − − − − − − − − −
2	feeding at 27.5° C.		− − + − − − − − − − − −
3	followed by 31		− − − − − − + − + + − −
4	days on immune		− − − + + −*
5	plants at 25° C.		− − − + + + − −*
6–15[b]			
16		27.5° C.	+ − + − − − − − − − − −
17			+ − + + + + + + + + + +*
18			+ − − − + −*
19			+ + − − − + + +*
20			− − + + − + + + + + − −*
21			+ + + + + + − +*
22			− − − − +*
23			− − − + + + +*
24			+ + + + + −*
25–30[b]			
31		32.5° C.	+ + + − + + + + +*
32			+ − − − + − −*
33			+ + +*
34			+ + + − − + + +*
35			− − − − + − − − −*
36			+ − + +*
37–45[b]			

[a] − indicates plant healthy; + indicates plant diseased.
[b] Insects Nos. 6–15, 25–30, and 37–45 did not transmit.
Asterisks indicate days on which insects died.

The preceding experiments demonstrated that not only the minimum incubation period varied at different temperatures, but that transmission did so as well. Frequent insect transmission occurred at temperatures ranging from 26° to 32.5° C., whereas both lower and higher temperatures proved unfavorable. The optimum for transmission seemed to coincide with the optimum for incubation.

Influence of a Temperature of 0° C. on the Retention and Incubation of the Virus in the Insect

The effect of a temperature of 0° C. on the retention of the virus was tested with a technique necessarily different from the preceding ones because no infection feedings or test feedings were possible at such a low temperature. Three hundred nonviruliferous nymphs of *Agallia constricta* were fed at 25° for 4 weeks on a source plant in order to render them infective. The viruliferous nymphs were then removed from the plants and placed in a glass tube immersed in a mixture of ice and water and kept in a refrigerator. After 6 weeks at 0° C., 30 surviving nymphs were tested in colonies of 10 insects on three healthy crimson clover plants at 25°, and the plants became infected during the first week of test feeding. The results indicated that the infectivity of the insect vector had not been influenced by the low temperature.

After it was found that the low temperature had no deleterious effect on the virus in the vector, the influence of this temperature was studied in relation to the incubation period. Three hundred nonviruliferous nymphs were fed for 7 days on a source plant at 25°, then removed and kept for 4-, 5-, and 6-week periods at 0° C. Less than 10 per cent of the insects survived the long treatment at 0° C. When tested on crimson clover plants at 25° they were unable to infect healthy plants during the first week after removal from the refrigerator, regardless of the length of the cold period. During subsequent weeks of testing they successfully transmitted the virus. It appears, therefore, that the incubation period was lengthened by a time equivalent to the duration of exposure to 0° C. Since it seems unlikely that differences in osmotic and diffusion processes at lower temperatures could account for the observed lengthening of the incubation period, the results suggest virus multiplication in the insect.

Incubation Period in Wounded and Nonwounded Vectors

Bawden (1), discussing the theory that certain plant viruses multiply in their insect vectors, came to the conclusion that the most probable explanation for the incubation period in this type of vector appears to be that it is the time necessary for the virus to penetrate the gut wall, pass through the blood, and enter the saliva so that it is in a position to be injected into plants. If this were the right explanation there should exist a way of speeding the incubation by mechanical injury of the vector during or after infection feeding. An experiment was made to find out whether there is a difference in the incubation period of punctured and nonpunctured insects. Virus-free insects were caged on two source plants for 7 days (Table 6). Fifty insects, divided into five colonies, were punctured on the third day of infection feeding, each insect being wounded by insect pins and then put back on the source plant for another 4 days. The control consisted of 50 insects from the same lot, not punctured and caged in five colonies on source plants for 7 days. The temperature throughout

the experiment was maintained at 27.5° C. The results showed no difference between the two groups. It is concluded, therefore, that puncturing had no effect on shortening the incubation period.

Incubation Period in Two Species of Vectors

All experiments described so far were performed with *Agallia constricta*. Black (3) found that it was a more efficient vector of wound-tumor virus than *Agalliopsis novella*. A preliminary experiment was carried out in which the incubation period of the virus in these two species was compared in the greenhouse, where the average temperature was lower than 25° C. One hundred *A. novella* and 110 *A. constricta* were fed simultaneously on the same source plant for 10 days, then tested in colonies of 10 insects. *A. novella* infected only one plant out of 221 plants tested, whereas *A. constricta* infected 37 out of 239 plants tested. These data

TABLE 6.—*Incubation period of wound-tumor virus in wounded and nonwounded Agallia constricta tested at 27.5° C.; colonies of 10 insects*

Treatment	Colony	Infection feeding	Transmission record[a] Days (from start of experiment) 14	16	18	20	22	24	26	28	30	32	34	36	38	40	42
Wounded on 3rd day of infection feeding	1	7 days	−	−	+	−	−	−	−	+	+	−	−	+	+	+	−
	2		−	−	−	+	+	+	+	−	−	−	−	−	+	−	−
	3		−	−	+	+	+	+	+	+	+	+	+	+	+	+	−
	4		−	−	+	−	+	+	+	+	+	+	+	+	−	+	+
	5		−	+	−	−	+	+	+	+	+	+	+	+	+	−	−
Not wounded	6	7 days	+	−	+	−	+	+	−	+	+	+	+	+	+	+	+
	7		−	−	−	−	+	+	+	+	+	+	+	−	+	+	+
	8		−	−	−	−	−	−	−	+	−	+	−	−	+	−	+
	9		−	−	−	+	−	−	+	+	+	+	+	+	+	−	+
	10		−	−	−	+	−	−	+	−	+	−	−	+	−	−	−
	Control		−	−	−	−	−	−	−	−	−	−	−	−	−	−	−

[a] − indicates plant healthy; + indicates plant diseased. All plants were healthy on the 8th, 10th, and 12th days.

clearly indicated a difference in transmission but were entirely inadequate to even indicate a difference in incubation. Therefore, in another experiment performed at 27.5°, five colonies of *A. novella* were compared with five colonies of *A. constricta*. Each colony consisted of 10 insects, and the infection feeding lasted 7 days. No difference was found in the efficiency of transmission, possibly because colonies were used instead of single insects. Neither was there a significant difference in the minimum incubation period in the two species.

Incubation Period in the Plant at Different Temperatures

It was found in preliminary experiments that in the greenhouse a minimum of at least 3 weeks elapsed before first symptoms of the disease could be detected in crimson clover plants. During the summer of 1947 most plants observed in the greenhouse developed symptoms only after 4

or 5 weeks. Experiments were designed to learn whether there is a relationship between the influence of temperature on the incubation period of the virus in the plant and in the insect vector. In the first experiment (Fig. 2, A), 60 crimson clover plants were inoculated at 25° by means of

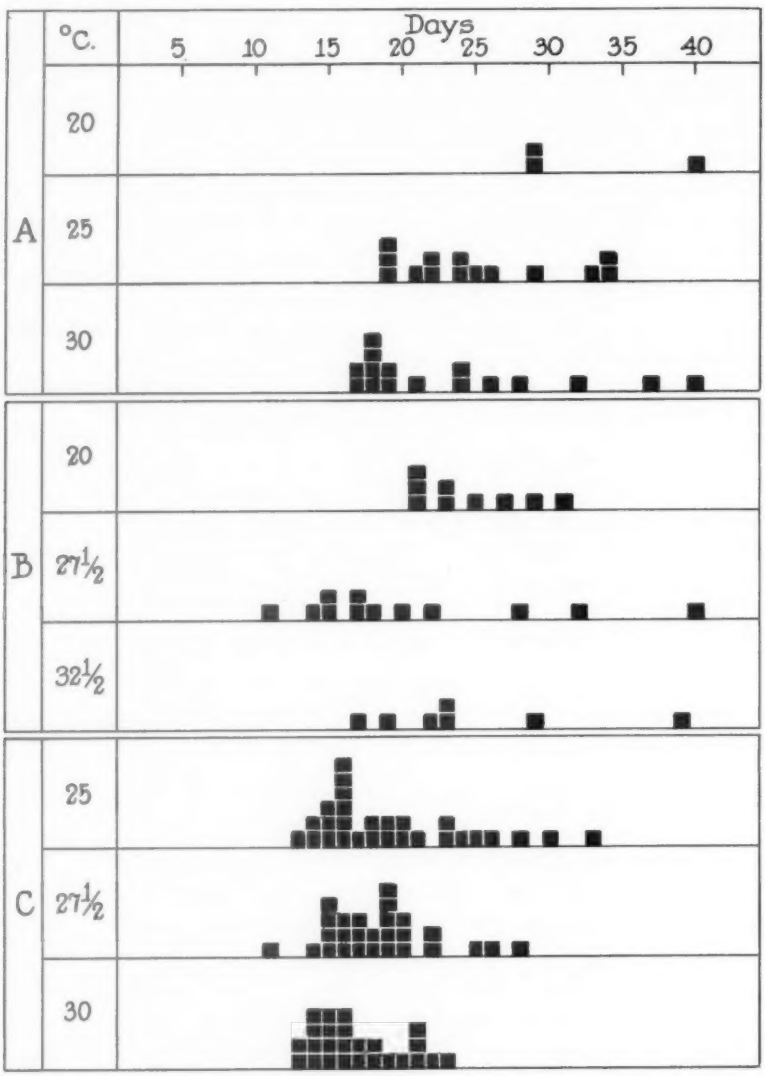

FIG. 2. Incubation period of wound-tumor virus in crimson clover plants (*Trifolium incarnatum* L.) at different temperatures. Black squares represent single plants with first symptoms of disease.

900 infective insects, 15 insects being caged on each plant for one day. Then the plants were divided at random into three groups and observed at 20°, 25°, and 30° for 6 weeks. The number of infected plants in each group was not so high as expected. The plants were 11 weeks old at the

start of the experiment and, as will be seen later, this may account, in part at least, for the comparatively poor results. At 20° the minimum incubation period was 28 days, at 25°, 17 days, and at 30°, 15 days. At 20° not more than three plants became diseased, compared with 14 and 16 plants at 25° and 30°, respectively. Plants kept at 20° for 6 weeks remained healthy when observed later in the greenhouse for an additional 8 weeks.

The next experiment was made with 150 plants. They were 5 weeks old at the start of the experiment and each plant was inoculated at 27.5° C. by five infective insects. After 24 hr. the insects were removed and the plants divided into three groups. Observations were made at 20°, 27.5°, and 32.5° C. The results (Fig. 2, B) showed a minimum incubation period of only 10 days at 27.5° and of only 20 days at 20°, which was considerably less than in the previous experiment at the same temperature. An explanation for the difference may be found in the age of the test plants.

In a third experiment, temperatures of 25°, 27°, and 30° C. were tested, 45 plants being used at each temperature (Fig. 2, C). The plants, 5 weeks old at the start of the experiment, were inoculated by colonies of 10 infective insects, caged for 24 hr. on the plants at 27.5° C. The experiment showed that there were no marked differences in the incubation periods over the temperature range of 25° to 30° C. The minimum incubation period of 10 days at 27.5° was confirmed. The equal number of plants with wound-tumor symptoms in each group (25–28 out of 45) showed that probably all plants into which the virus had been introduced developed symptoms of the disease. Considering the distribution of incubation periods it could be deduced that 30° C. was optimal, since only 11 days elapsed between the development of symptoms in the plant with the shortest incubation period and that with the longest; at 27.5° C. this period was 16 days and at 25°, 21 days. On the other hand, under the conditions of the experiment, 30° inhibited seriously the growth of the plants even during the short run of the test. Therefore probably 27.5° will be more suitable for future experimental purposes.

Incubation Period and Susceptibility in Relation to Age of Plants

The incubation period of the virus was tested on four groups of 10 crimson clover plants of different ages. The susceptibility to infection was also observed. The test feeding lasted 6 days, each plant being inoculated by a colony of 15 infective insects. The youngest plants were 3 weeks old at the start of the experiment, the oldest 13 weeks. It was found (Fig. 3) that older plants were less susceptible to the virus. The longer incubation period of the virus in older plants probably explains the difference in results shown in figure 2.

Incubation Periods in Vectors Injected with Different Dilutions of Virus

If the incubation period is mainly the time required for multiplication of virus to an infective concentration in the vector (2, 6, 9, 10, 14, 15), a virus that multiplies rapidly should have a short incubation period and a virus that multiplies slowly should have a long one (17). As the wound-tumor virus was found to have one of the longest incubation periods reported for a plant virus, it was believed that the virus multiplies slowly. If that were so, differences could perhaps be detected in the incubation period of insects injected with different dilutions of virus. Recently the mechanical transmission of the virus by means of injections was described (20) and it was also reported that the virus had an incubation period in injected insects. The technique will be briefly described. Infective insects were ground at 0° C. with an equal weight of 0.25M NaCl. The suspension was centrifuged at 3500 r.p.m. for 5 min. and the supernatant diluted in 0.25M NaCl to 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4} and used for injection. The solution was drawn up into a fine glass capillary and a small amount

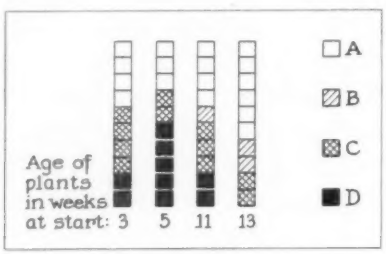

FIG. 3. Incubation period of wound-tumor virus and susceptibility to infection in crimson clover plants at different ages. One square represents one plant: A, not infected; B, incubation completed in more than 6 weeks; C, incubation completed in 5 weeks; D, incubation completed in less than 4 weeks.

forced into the insect through a puncture made in the abdomen with the glass capillary. All insects were injected in the nymphal stage and kept at 27.5° during the test feeding. It was found that both nymphs and adult males and females became infective and transmitted the disease. The injection of different dilutions of the virus into insects resulted in incubation periods of different lengths (Table 7). A dilution of 10^{-1} gave a surprisingly short minimum incubation period of only 7 days, whereas dilutions of 10^{-2} and 10^{-3} resulted in 11-day minimum periods and 10^{-4} required a 19-day minimum period. The results were compared with a group of 50 insects that acquired the virus by feeding on a source plant for 7 days and were tested in five colonies at 27.5° simultaneously with the injected insects (Table 2, C); in table 7 their transmission record is presented in summarized form. The minimum incubation period in this group was 13 days. In various other experiments insects that acquired virus by feeding were tested at different temperatures. In no instance was a shorter incubation period than 13 days found. Therefore, this

minimum period was firmly established for insects that acquired the virus by feeding. The occurrence of three incubation periods shorter than this in the first experiment with injected insects therefore indicated a real difference. The data are interpreted as meaning that when high concentrations of virus are injected, a shorter time than normal is required for the virus to reach an infective concentration. Injections with lower concentrations of virus require more time to reach the same level.

The experiment also showed the persistent transmission of the virus by injected vectors. The insects infected healthy plants until they died. It is very likely that the virus persisted also in insects injected with a dilution of 10^{-1}, although this cannot be seen from the results, in table 7. The failure to determine this was due to the death of the transmitting insects.

TABLE 7.—*Incubation period of wound-tumor virus in Agallia constricta at 27.5° C.; insects inoculated by injection and by feeding*

Means of infection	Number of insects tested	Transmission record[a] Days (from start of experiment) 1–7	8	10	12	14	16	18	20	22	24	26	28	30	32	34	36	38	40	Days of incubation period
Injection[b]																				
10^{-1}	25	−	+	−	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	7
10^{-2}	32	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	11
10^{-3}	30	−	−	−	+	+	+	+	+	+	−	+	+	+	−	+	−			11
10^{-4}	38	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	−	+	19
Feeding[c]	50	−	−	−	−	+	−	+	+	+	+	+	+	+	+	+	+	+	+	13
Control	28	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	

[a] − indicates plant healthy; + indicates plant diseased.
[b] Juice from infective insects diluted and injected into vectors at nymphal stage.
[c] Summarized transmission record of five colonies (Table 2,C) tested simultaneously with the injected insects. Altogether in various experiments 1,210 insects inoculated by feeding were tested at different temperatures; the minimum incubation period was never below 13 days.

Not all injected insects transmitted the virus, and as their number decreased considerably during the test feeding, all the potential vectors may have died by the 26th day. The group injected with the 10^{-1} dilution was, incidentally, the smallest one, and it was reduced to but four insects after the 26th day. The persistence of the virus has also been tested in single insects that acquired the virus by feeding. In one instance an infective female was tested for 81 days, until she died; during this time she transmitted the virus with but few plants escaping infection. These results (obtained at 27.5° C.) indicated that the manner in which the insects acquired the virus had no influence on its persistence.

Occurrence of Virus-Free Cuttings from Infected Plants

The effect of controlled temperature on disease expression was studied on cuttings of sweet clover plants (*Melilotus* sp.). It was found by Black (5) that different clones obtained from commercial seed of sweet clover differ greatly in their symptom expression. Some develop large tumors on roots and stems, others only on the roots; still others were found to develop only very small or very few tumors on the roots.

In the first experiments, cuttings from different clones were used at 16°, 26°, and 35° C. It was found that at 35° almost all the plants died, whether diseased or healthy. During the long period of the test (90 days), 16° C. proved to be best for plant growth. Not all cuttings inoculated by means of insects became infected at 16° and 26°, probably because of the failure of some insect colonies to infect them during the 7 days of test feeding. But included in the experiment were some cuttings from plants infected in 1945 with the wound-tumor virus; the roots of these plants, designated as clones C8, C9, C10, C11 (Black, unpublished data), were so tumorous that the plants grew very poorly and the clones had been maintained for three years by grafting scions onto well-rooted, healthy plants of medium size. Subsequently, the grafted plants were pruned in such a way that the top of the plant consisted of scion material only. In propagating the plants as clones in this manner, scions were selected from the tips of the branches where no tumors had yet developed. In spite of this the grafted scions had always developed tumors and the roots of the healthy stocks also eventually became tumorous. When cuttings of these clones were used in the experiments it was found that stem and root tumors developed well at 26° C., similarly to the tumors in recently inoculated plants. As was to be expected, tumor development started later at 16° than at 26° C. However, after 3 months at 26° C. some cuttings of C8, C10, and C11 remained healthy in appearance. They were examined again after 5 additional months in a greenhouse, but no root or stem tumors were found. The plants were apparently free from the virus (19).

Whether this was the right explanation for the lack of tumors or whether the plants had become masked carriers still was undetermined. It has been demonstrated for other plant virus diseases (22) that acquired immunity is always connected with the presence of the virus in the plant. A plant that has acquired immunity to a virus disease usually cannot be infected with strains of the same virus. Therefore, cuttings from symptomless clones C8, C10, and C11 were inoculated by means of infective insects, and an equal number of noninoculated cuttings from the same clones was included as controls. In all cases, characteristic root and stem tumors resulted from these reinoculations, whereas all controls remained symptomless. Because reinoculation produced infection, it was concluded that the symptomless plants obtained from these clones were virus-free.

When the occurrence of virus-free cuttings of C8, etc., was proved, another very susceptible clone, B21-a, infected with wound-tumor virus and propagated for 2 years in the same manner, was also used for an experiment. A single plant was chosen with tumors already developed on the lower part of the stem, and 30 cuttings were rooted. After 4 weeks they were placed for 60 days in groups of 10 plants at 20°, 27.5°, and 32.5° C. Fifty per cent of the plants at all three temperatures remained healthy.

The conditions influencing the occurrence of virus-free cuttings have not yet been defined. The data indicate that under certain conditions

the virus may not invade the tips of young branches, and that cuttings from such tips are therefore healthy. If this is the explanation of virus-free cuttings in these experiments, the case would be similar to that reported for spotted wilt in dahlia by Holmes (12).

DISCUSSION

There are some immediate practical implications of the temperature studies. It is clear that ordinary greenhouse conditions would give widely different results with wound tumor, depending on temperature variations. In particular, temperature of about 20° C. (68° F.) would greatly lengthen the incubation period in the insect and seriously reduce the frequency of transmission, as well as prolong the incubation period in the plant. To obtain results in future experiments in the shortest period of time, greenhouse temperature should be kept close to 27° C. (80° F.). This would reduce the length of time required for experiments by almost half.

The experimental results suggest the possibility that a mean difference of only 5° C. may sometimes account in nature for a severe or mild outbreak of virus diseases. In different months or years such a comparatively small difference in mean temperature, at the critical time when plants are most susceptible to virus infection, might either prevent or favor the successful transmission by a vector.

The results of temperature studies may also add some information concerning the nature of incubation periods of viruses in their insect vectors. Many explanations have been put forward to account for this period. It has been suggested that passage through the vector may change the infecting properties of the virus in much the same way as the infecting powers of the spores of some rust fungi are altered by their hosts. No evidence in favor of the existence of different stages in plant viruses has been reported, but Mosley and Wyckoff (21) described filamentous stages in influenza virus, and more recently Chu, Dawson, and Elford (8) reported very long filamentous stages in influenza virus, suggestive of a complicated life cycle in this virus. There is no evidence for such a change in the wound-tumor virus; neither can the life-cycle hypothesis explain the lengthening of incubation periods in insects injected with higher dilutions of virus. Wound-tumor virus is readily transmitted from plant to plant by grafting and from insect to insect by injection. Also, it has recently been shown that the virus can be transmitted mechanically from plant extracts to vectors by injection (7).

Another explanation for the incubation period is that the vector, while feeding on infected plants, acquires an amount of virus insufficient for transmission. The incubation period is regarded as the time required for this amount to multiply in the insect to infective concentrations, plus the time required for the virus to reach points in the insect's body where it can be injected into plants.

Considerable evidence for multiplication of plant viruses in insect vec-

tors has been presented (2, 6, 9, 10, 14, 15). In view of such evidence for viruses having long incubation periods in leafhoppers, interpretation of the results of the present study on the basis of multiplication of the virus in its insect carrier seems most likely to be the correct one. Therefore, the study of the influence of temperature on incubation periods seems important, also, as it may add to our understanding of the process of virus multiplication.

The incubation periods of wound-tumor virus in plants at different temperatures showed a rough relationship to those in insects at the same temperatures. The lengths of both vary inversely with temperature up to about 27.5° C., above which plants were injured. The correlation is in harmony with the evidence that some plant viruses multiply in leafhoppers as well as in plants.

In 1948, when these experiments on wound tumor were still in progress, a similar relationship between the incubation periods of plant viruses in plants and insects was reported by Kunkel (17), who found that the incubation period of corn stunt virus in plants and vectors during autumn was shorter than during colder winter days in the greenhouse. In the same paper the author reported that under highly favorable conditions of temperature the virus of aster yellows was found to have an incubation period of 9 days, both in the vector and in the plant. Kunkel also pointed out that the lengths of incubation periods reported in a number of instances under ordinary greenhouse conditions are sufficiently accurate to show that the correlation between the length of the incubation period in the plant and in the insect holds for several other viruses.

The existence of an incubation period in injected vectors of virus diseases was observed by Storey in 1933 and by Black in 1941. Storey (24) found a difference in the behavior of injected insects in the transmission of maize streak. Inoculated insects caused infections more irregularly and ultimately became noninfective, whereas those acquiring virus by feeding usually continued to cause infections up to the time of their death. Black (2) found that vectors of aster yellows injected with juices from infective insects had an incubation period and the virus persisted as long as the insects lived. Injections of different dilutions of juices from insects carrying wound-tumor virus into nonviruliferous vectors resulted in a minimum incubation period of 7 days at a dilution of 10^{-1}. This period was considerably shorter than those resulting after infection feeding. The data show that high dilutions need longer incubation than low dilutions and suggest multiplication, since a small amount of virus might be expected to require more time than a large amount to render an insect infective. However, other explanations are possible. The shorter incubation period in injected insects could also be explained by the direct introduction of virus into the blood. Storey (25) concluded from experiments on *Cicadulina mbila* that the streak virus, entering the intestine by mouth, passed through the intestinal wall into the blood of the vector. If we assume

that the passage of the intestinal wall accounts for most of the incubation period, a considerable shortening should be observed in all injected insects even at high dilutions and also in insects punctured during their infection feeding. That wounding itself has no such influence was shown in the experiment with punctured and nonpunctured insects. It also is difficult to see how the explanation for the incubation period, as being due merely to the virus penetration of the gut wall, passing through the blood and entering the saliva (1), could explain a correlation between the length of incubation periods of plant viruses in the host and in the vector.

In the case of wound-tumor virus the experiments showed persistent transmission by injected insects as well as by insects inoculated by feeding. If persistent transmission were a result of accumulation of wound-tumor virus and not due to multiplication, the virus should not persist in insects injected with high dilutions. The fact that persistence was observed with high dilutions suggests multiplication of the virus in injected insects.

Conditions favoring the occurrence of virus-free cuttings have not yet been established. The phenomenon is important from both a practical and a theoretical point of view. From a practical standpoint clones of striking or unusual tumor response, previously available only in the infected state, have now been obtained in the virus-free condition as well. From the theoretical viewpoint it is important to investigate this phenomenon further to ascertain, if possible, what conditions give rise to virus-free plants and whether they derive from cells that have always been virus-free or from cells in which the virus has been destroyed or lost. The recognition of conditions favoring this phenomenon may permit more extensive use of healthy cuttings from plants systemically infected with wound-tumor virus.

SUMMARY

The influence of temperature on the incubation and transmission of the wound-tumor virus was studied under controlled conditions of light and temperature. Alternating periods of 16 hr. of light and 8 hr. of darkness were used in all experiments. The number of tested insects exceeded 1300; more than 6000 plants were used in the experiments.

The shortest incubation period of the virus in the insect vector was found to be 13 to 15 days at temperatures ranging from 25° C. to 32.5° C. This is one of the longest incubation periods reported for plant viruses, none others as yet having been determined at different constant temperatures. Lower temperatures of 16° and 20° C. resulted in longer incubation periods (30 days). The long incubation period at low temperatures was found to be real and not caused by poor transmission. A temperature of 0° C. lengthened the incubation period by a time equivalent to the duration of exposure to 0° C. At 37° C. no transmission occurred and therefore no incubation periods were observed. Transmission at 16° and

20° C. was poor but returned to normal when the insects were tested at a suitable temperature.

Minimum incubation periods of the virus in two vectors, *Agallia constricta* and *Agalliopsis novella*, at 27.5° C. did not differ.

Puncturing the insects by means of insect pins on the third day of a 7-day infection feeding period did not shorten the incubation period.

Nymphs as well as adults of both sexes transmitted the wound-tumor virus, both nymphs and adults being able to acquire the virus. No difference in the incubation period was observed between nymphs and adults.

The length of the incubation period of the disease in plants at a given temperature seems related to that in the insect vector. This is attributed to the probability that in both plant and insect the incubation period is an interval required mainly for the multiplication of the virus.

The incubation period in old plants was longer than in young plants and their susceptibility to infection was lower.

Different dilutions of virus from viruliferous insects resulted in different incubation periods in the injected insects. With a dilution of 10^{-1} the incubation period was only 7 days, as compared to 13 days in the case of insects that acquired the virus by feeding. Wih dilutions of 10^{-2} and 10^{-3} the incubation period was 11 days; and with 10^{-4}, a minimum of 19 days. These results are interpreted to mean that high dilutions need a longer incubation period than low dilutions, because the multiplication of a small amount of virus would take a longer time to render the insect infective than multiplication of a somewhat larger amount.

The virus was found to persist in the vector until death, irrespective of whether it was acquired by feeding or by injection.

Virus-free plants were obtained from sweet-clover cuttings taken from clones infected with the virus for several years.

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ENVIRONMENT AND HOST RESISTANCE IN RELATION TO CUCUMBER SCAB

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(Accepted for publication July 10, 1950)

Since Arthur (1) described *Cladosporium cucumerinum* Ell. & Arth. on the fruits of cucumber in 1889, the fungus has continued to incite the disease known as scab or spot rot of this crop in northern States from Wisconsin to Maine, in Canada, and in northwestern European countries. In the United States the disease is most severe in the northern New England States and in a relatively limited area of Wisconsin and Michigan, centering in northern counties adjacent to the Great Lakes. In Wisconsin the disease appears regularly, beginning in the last half of July or early in August, in Brown and Oconto Counties, which are adjacent to Green Bay, and it often increases rapidly to become a destructive epidemic. Within this restricted area it is most severe in fields located in creek bottoms or where surrounding stands of timber restrict air circulation. The disease is enhanced by periods of cool nights and night fogs in low areas. In other counties of the northern half of Wisconsin scab is relatively rare and spasmodic; in the southern half of the State it is practically absent.

The present investigation is concerned with 1) environal factors that contribute to the development of the disease; 2) the influence of environment on the expression of host resistance; and 3) the inheritance of host resistance.

RELATION OF TEMPERATURE TO DISEASE DEVELOPMENT

In a large number of experiments at controlled greenhouse temperatures, pot-grown plants were inoculated at ages varying from first-leaf to tenth-leaf stage. Some were left in the moist chambers for the duration of the experiment; others were removed after 48 hr. The moist chambers were controlled at 13°, 17°, 21°, and 25° C.

In susceptible varieties the disease appeared first on the youngest tissue of the growing bud and, depending upon the influence of environment, extended down the stem, involving older leaves successively. At 25° C. the first signs usually appeared on about the fifth day; at successively lower temperatures the intervals lengthened. The rate of progress, however, shifted promptly to an optimum at 21° or 17° C. The age of the plant had a consistent effect upon the relative progress of the disease at the last two temperatures. In plants inoculated at the first-leaf stage the disease progressed most rapidly at 21°; at the second-leaf stage and older, 17° was clearly the optimum if growing conditions were favorable (Fig. 1). In

[1] The author is indebted to Dr. D. C. Smith for advice in preparation of the manuscript.

Mrs. Frances McComb assisted in the execution of a portion of the work reported.

older plants, pot-bound and growing slowly, the disease was sometimes most damaging at 13°.

It has been shown by Wiant (10), Pfältzer (7), and Muyzenberg (6) that the optimum for growth of the pathogen on potato-dextrose agar was about 21° C. This was confirmed in the present studies. This might be expected to be the optimum for the disease if the host reaction was not affected by temperature. The growth of cucumber plants, under these greenhouse conditions, is best at about 25° and it decreases with temperature to very little growth at 13°. The effect of host growth on disease development was very noticeable over the temperature range of 13° to 25°. Although the first signs appeared at 25° followed closely by signs at 21°,

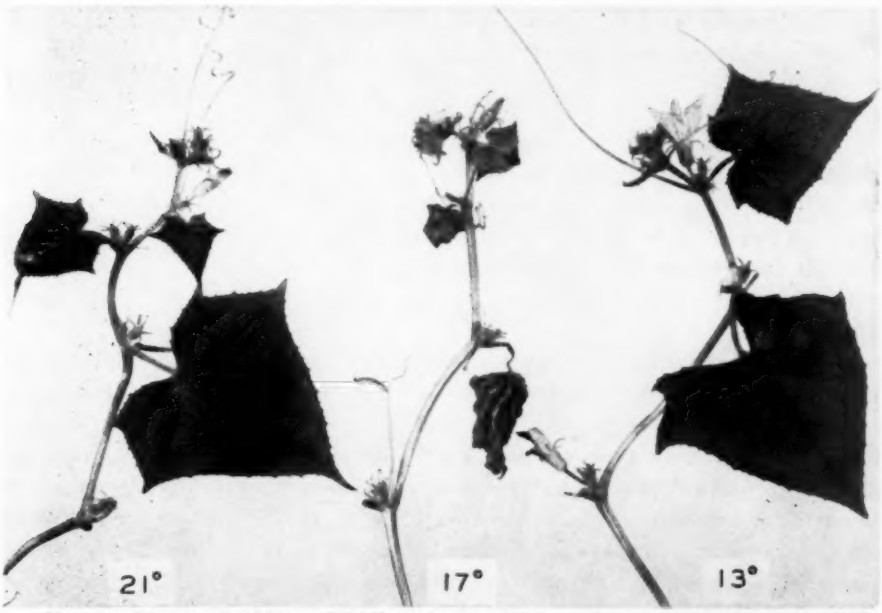

FIG. 1. Plants of Chicago Pickling (susceptible) variety inoculated in the sixth-leaf stage and kept in moist chambers at the temperatures (°C.) indicated for 9 days. Note necrosis of young leaves at 17° and definite but less severe infection at 13°. Signs of very slight infection at 21° are not evident in the photograph.

the progress of lesions at these two temperatures was soon retarded and as a rule the lesions were promptly corked off into tan-colored cankers, while the growing point or a bud immediately below it continued to develop. At 17° growth of the host was retarded much more in proportion to the growth of the fungus, and lesions continued to enlarge and progress down petioles and stems with little or no canker formation. As a result, in plants beyond the second-leaf stage the disease progressed most rapidly at 17°. The very slow growth of the host at 13°, together with the fairly active growth of the fungus, resulted eventually in about as much damage as at 17°. In plants inoculated at the very early first-leaf stage the growth response of the host was not effective enough to ward off disease damage

at 21° and below; the disease was actually as severe or slightly more so at 21° than at 17°. At 25° in first-leaf plants the growth response was prompt enough to retard disease development distinctly.

When a temperature of 17° was maintained in the moist chamber and plants in the second-leaf stage were used, penetration took place in as little as 6 hr., but the extent of infection increased with duration of the inoculation period up to 30 hr. When inoculated plants were placed in the moist chamber for 2-, 4-, and 6-hr. intervals on successive days, slight infection occurred after two successive daily 2-hr. periods and severity increased with 4-hr. and 6-hr. daily exposures in the moist chamber. It was concluded that with optimum humidity and temperature, infection could take place in two or three successive nights in which high humidity was maintained for 2 hr. Increase in the length of the daily humid period, however, increased severity of the disease.

RELATION OF ENVIRONMENT TO EXPRESSION OF RESISTANCE

In the study of inheritance of resistance it was important to know whether that character is expressed at an early stage in host development and whether it is sufficiently constant under optimum environment for disease development to permit accurate classification of resistant and susceptible individuals into discontinuous classes. Studies were carried out with Maine 2 variety developed at the Maine Agricultural Experiment Station (3).

When plants of the resistant variety were included in the temperature studies previously described they maintained a high degree of resistance (Fig. 2, A). Infection was not precluded, however, and water-soaked lesions appeared on the youngest leaves, or marginal necrosis became the chief sign. However, at no temperature was infection severe enough to result in large stem lesions or cankers (Fig. 2, D). Young infected leaves continued to grow and as the lesions became delimited by healthy growing tissue various degrees of malformation or raggedness of the leaves developed (Fig. 2, B). At optimum temperature for disease development signs on resistant plants were in marked contrast with the rapid killing of the growing point and stem down to the cotyledonary node in susceptible plants. If temperatures from 20° to 25° prevailed, the contrast between resistant and susceptible individuals was greatly reduced because of the tendency of the latter to cork out incipient lesions and to produce new healthy tissues and organs.

It was clear from this study that the resistance in Maine 2 was well defined in young seedlings and that it was essential to provide temperatures around 17° C. in order to secure an adequate differential test and to avoid the escape of susceptible individuals.

INHERITANCE OF RESISTANCE

Since the ultimate purpose of the investigation was to develop a scab-resistant variety suited to the northern Wisconsin pickle industry, it was

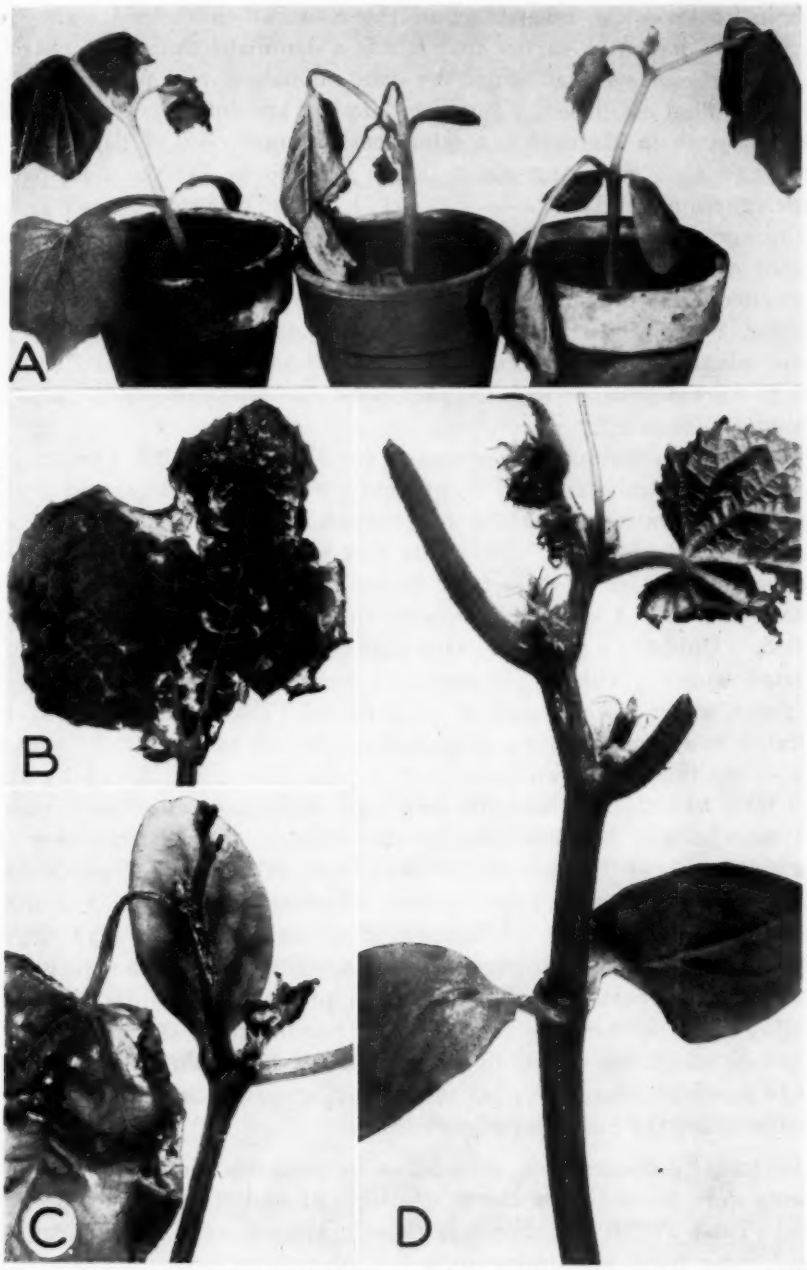

FIG. 2. A. Resistant plants of Maine 2 and susceptible plant of Chicago Pickling (center) inoculated in the third-leaf stage and held in the moist chamber at 17° C. Note killing of stem of susceptible plant and marginal burning of youngest leaves in resistant plants. B. Leaf of Maine 2 (resistant) plant on which small lesions appeared after inoculation at 17°, but in which the leaf continued to produce malformed growth. C, D. Susceptible National Pickling (C) and resistant Maine 2 (D) inoculated in the fourth-leaf stage and held in the moist chamber at 17° for 13 days. Note killing of stem of susceptible plant and marginal necrosis of youngest leaf, and small stem canker in resistant plant.

essential to know the inheritance of the resistant character. Bailey and Burgess (4) indicated earlier that it was a dominant character controlled by a single gene pair, although the data published were not sufficient to warrant a final conclusion. Further data are presented herewith to show that resistance in Maine 2 is a monogenic character. The data were derived from tests on young plants in the greenhouse, for the most part at temperatures about 17° C.

Chicago Pickling, National Pickling, Ohio 31, and Stays Green were selected as susceptible parents. In repeated tests these were uniformly susceptible. F_1 progenies from crosses between Maine 2 and Chicago Pickling, Stays Green, and Ohio 31, as well as between Maine 2 and F_1 hybrid plants of Chicago Pickling × Ohio 31 and of National Pickling × Ohio 31 were inoculated with *Cladosporium cucumerinum*. Resistance was completely dominant.

Sixteen F_1 individuals from crosses involving susceptible parents from four varieties were selfed and F_2 progenies were tested. Fourteen of these segregated at ratios which did not deviate significantly from that of 3 resistant to 1 susceptible. Two progenies had a highly significant deviation from 3:1. One of these from Maine 2 × Chicago Pickling upon retest conformed to a 3:1 ratio. There were only two progenies from (National Pickling × Ohio 31) × Maine 2. One segregated closely to a 3:1, the other deviated widely. This would naturally raise the question as to possible difference in the genetic makeup of National Pickling and Ohio 31 with reference to resistance and susceptibility. In this connection it should be pointed out that, as shown in table 1, F_2 progenies from sibs of F_1 plants from Ohio 31 × Maine 2 and the reciprocal segregated close to 3 resistant to 1 susceptible. Furthermore, as shown later, the F_3 progenies from progeny No. 1 of (National Pickling × Ohio 31) × Maine 2 supported a monohybrid hypothesis of inheritance of resistance. In table 2 the F_1 from a cross of (Maine 2 × National Pickling) × Ohio 31 gave the 1:1 segregation expected with that hypothesis. Moreover in the work which followed that reported here, a back-cross program of Maine 2 × National Pickling, with the latter as the recurrent parent, gave segregation in each back-cross which was a close fit to the expected 1:1 ratio. It seems justifiable to conclude, therefore, that the F_2 data strongly support a monogenic hypothesis for the inheritance of resistance.

Certain F_1 plants from the crosses between resistant and susceptible parents were crossed with susceptible Ohio 31 and the resulting progenies tested (Table 2). It was necessary to make these tests in midsummer when greenhouses were not under sufficient control to prevent temperatures rising frequently to 25° C. While the ratios in no case deviated significantly from the expected 1:1, in each case the population of diseased plants was lower than that of healthy plants. It is probable that this was attributable in part to a few escapes resulting from temperatures above the optimum for disease development.

TABLE 1.—*Reaction of F_2 progenies from crosses between resistant and susceptible varieties when inoculated in the seedling stage with Cladosporium cucumerinum*

Cross	Progeny No.	Classification of plants		
		Healthy	Diseased	P value 3:1 ratio
Chicago Pickling × Maine 2	1	10	3	0.90–0.80
Do	2	13	4	0.90–0.80
Do	3	12	1	0.20–0.10
Do	4	5	5	0.10–0.05
Maine 2 × Chicago Pickling	1	13	6	0.70–0.50
Do	2	9	1	0.30–0.20
Do	3	49	21	0.50–0.30
Do	4	49	17	0.90–0.80
Do	5	30	23	<0.01**
Do	5	19	4	0.50–0.30
Do	6	13	4	0.90–0.80
Do	7	15	4	0.70–0.50
Do	8	13	5	0.80–0.70
Maine 2 × Stays Green	1	14	2	0.30–0.20
Do	2	16	5	0.90–0.80
(National Pickling × Ohio 31, F_1) × Maine 2	1	48	17	0.90–0.80
	2	16	14	<0.01**
Maine 2 × Ohio 31	[a]	32	8	0.50–0.30
Ohio 31 × Maine 2	[a]	29	5	0.20–0.10
(Chicago Pickling × Ohio 31, F_1) × Maine 2	[a]	119	47	0.50–0.30

[a] Progeny from sibbed F_1 plants.
** Highly significant.

One hundred and thirteen F_2 plants were tested as to their genotype by inoculating self or back-cross progenies. These came from four crosses, two of which were reciprocals (Table 3). In three crosses the susceptible segregates in the F_2 progenies were eliminated and only survivors of the disease test were carried on to produce seed for progeny tests. In the data (Table 3) for each cross there was a fair approximation to the expected 1:2 or 1:2:1 ratios.

Certain progenies, not included in table 3, did not conform to expected ratios. In Chicago Pickling × Maine 2 one self progeny had 23 healthy

TABLE 2.—*Reaction of progenies from crosses between F_1 plants from resistant × susceptible parents, and the susceptible variety Ohio 31, when inoculated with Cladosporium cucumerinum*

Cross	Healthy plants	Diseased plants	P value 1:1 ratio
	Number	*Number*	
(Maine 2 × Stays Green) × Ohio 31	55	40	0.20–0.10
Do	41	27	0.10–0.05
Do	14	13	0.90–0.80
(Maine 2 × National Pickling) × Ohio 31	23	17	0.50–0.30
(Chicago Pickling × Maine 2) × Ohio 31	30	24	0.50–0.30
Do	56	40	0.20–0.10
Do	17	13	0.50–0.30

plants and one diseased plant. This may have been a case where several susceptible plants escaped in a heterozygous progeny, or it may have been a homozygous resistant progeny in which a susceptible plant was included by mistake. In the reciprocal cross one segregating progeny had eight diseased and eight healthy plants, a ratio that deviated too widely from 3 to 1 to be included in that class. In the cross Maine 2 × Stays Green, one segregating progeny had four healthy and six diseased plants. This again did not conform. In the last cross listed in table 3, one self progeny had six healthy and 15 diseased plants; one had one healthy and 17 diseased plants; one had 32 healthy and one diseased plants. While no entirely satisfactory explanation can be offered for these cases, it may be postulated that, if there were a small number of susceptible escapes in each of these instances, two of the last three progenies mentioned would probably have been found to be homozygous susceptible and the other one to be segregating close to 3 resistant to 1 susceptible. This would have changed the figures for the last cross listed in table 3 to 8 resistant, 11 heterozygous, and 9 susceptible. These figures would still fit fairly well within the expected 1 : 2 : 1 ratio (P = 0.70–0.50).

TABLE 3.—*Classification of F_2 plants on the basis of progeny tests from crosses between resistant and susceptible plants when inoculated with Cladosporium cucumerinum*

Cross	Resistant	Heterozygous	Susceptible	P value 1 : 2 or 1 : 2 : 1 ratios
Chicago Pickling × Maine 2	9	11	[a]	0.30–0.20
Main 2 × Chicago Pickling	16	22	[a]	0.30–0.20
Maine 2 × Stays Green	9	15	[a]	0.70–0.50
(National Pickling × Ohio 31, F_1) × Maine 2	8	10	7	0.70–0.50

[a] The susceptible segregates of the F_2 progeny were not propagated.

All of the inoculations reported above were made with an isolate of *C. cucumerinum* secured from diseased plants collected near Green Bay, Wisconsin. Parallel tests made later with resistant, susceptible, and segregating progenies inoculated with an isolate from Maine, provided by Dr. Donald Folsom, yielded similar results.

DISCUSSION

The appearance of scab in severe form in Wisconsin is confined in most seasons to a relatively limited area in the northeastern portion of the State, where low night temperatures and heavy dews or protracted foggy weather prevail after midseason. The organism has a relatively low optimum for growth on agar (ca. 21° C.) and sporulates profusely on foliage only in high relative humidity. Under controlled conditions the optimum for disease development is about 17° C. This is not in accord with the reports of Schultz (8) and Behr (5) in Germany who give an optimum of 25° C. This discrepancy may be due in part to a difference in interpre-

tation. In the present study the incubation period was shorter at 25° than at lower temperatures. However, the most severe disease development following the initial appearance of symptoms was usually at 17°, rarely at 21°, and never at 25°. It is evident that the host-parasite interaction is influenced differently by temperature than is host or parasite separately. Plants of the varieties studied grow most rapidly at 25°, but at this temperature and at 21° the cicatrization reaction of the host tissue in the vicinity of infection foci results in rapid corking-off of lesions. At 17°, where growth of both host and parasite is reduced little and where no corking-off occurs, the pathogen advances rapidly through the tissue of susceptible varieties. At 13°, damage is similar but much slower in development. The experimental results coincide in general with the field observation.

The variety screening tests of Bailey and Burgess (2, 4), Schultz and Röder (9), and Schultz (8) show that most varieties of cucumber are susceptible. Maine 2, one of the resistant varieties developed by Bailey (3), was shown in this study to be consistently resistant in the seedling stage under the most favorable environmental conditions for penetration and infection. With favorable temperature and humidity provided, it is possible to distinguish individuals with Maine 2 type of resistance from susceptible ones and to set up two discontinuous classes. The resistance of Maine 2 was completely dominant over susceptibility in crosses with the susceptible varieties, National Pickling, Chicago Pickling, Stays Green, and Ohio 31. The data secured from F_2 progenies, from back-crosses of F_1 progenies to a susceptible parent, and from F_3 progenies substantiate the hypothesis that the resistance and susceptibility to the scab fungus in these varieties are controlled by a single gene pair. While some divergent ratios were obtained, these were not considered to nullify the conclusion indicated. Progenies resistant in greenhouse tests remained completely free from symptoms when grown under field conditions which favored epidemic development on susceptible varieties. Isolates of the pathogen from Wisconsin and from Maine had parallel effects on resistant and susceptible progenies.

SUMMARY

Cucumber scab occurs in epidemic form in Wisconsin only in those areas where cool weather with frequent night fogs prevails after midseason. The optimum temperature for disease development is about 17° C. At 21° and above, with the susceptible pickle varieties studied, the host cicatrizes the incipient lesions rapidly, with relatively little damage to the plant resulting. Under optimum environment, penetration of the resistant variety Maine 2 occurs and delimited local lesions develop without the rapid stem invasion which prevails in susceptible varieties. With optimum temperature and humidity, resistant and susceptible plants can be divided into discontinuous classes in the young seedling stage. With the use of this method it was possible to analyze segregating progenies with a

high degree of accuracy, and by this means it was shown that the resistance of Maine 2 and the susceptibility of the other varieties used are controlled by a single gene pair.

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VERTICILLIUM WILT OF BRAMBLE FRUITS WITH SPECIAL REFERENCE TO RUBUS URSINUS DERIVATIVES

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(Accepted for publication July 12, 1950)

Bramble fruit growers of California have sustained heavy losses in recent years from Verticillium wilt caused by the fungus *Verticillium albo-atrum* Rke. & Bert. (20). The factors mainly responsible for these losses have been the high degree of susceptibility of the two leading bramble varieties, Boysen and Young, and the utilization during the recent expansion of the industry of land planted previously to tomato, potato, or cotton. Almost without exception, land that has been planted to one or more of these crops is infested to a fairly high degree with the wilt Verticillium. Although the disease is present in all of the bramble-growing regions of California, heaviest losses have occurred in the coastal counties. The years 1946–1948 inclusive were characterized to an unusual degree, along the coast, by cool and overcast conditions in the spring, followed in early summer by an abrupt onset of hot weather. This combination of factors appears to favor Verticillium wilt.

The disease has been studied extensively in raspberries (5, 9, 11, 22), but has not been adequately treated for brambles of the blackberry type and their allies. Lawrence (11), who in 1912 described the blue-stem disease (Verticillium wilt) of raspberry, reported the isolation of *Acrostalagmus caulophagus* sp. nov. from the Snyder blackberry, a probable *R. alleghenisensis* derivative. *Acrostalagmus caulophagus* is considered to be a synonym of *Verticillium albo-atrum* Rke. & Bert. (10, 14). Adams (1, p. 373) in 1923 cited Shear's observations that blackberries were killed quickly by blue-stem in New York. In the following year Orton and Wood (13, p. 130) reported that Verticillium wilt was severe in all of the blackberry-growing regions of California. Neither Orton and Wood nor Adams mentioned the varieties affected. In 1927 Archer (2, p. 85) cited Rudolph's observations that the black and red forms of the Logan variety were resistant to Verticillium wilt. Rudolph (14) mentioned the disease in the Young and Lucretia varieties in 1931. Zeller's study of Verticillium wilt of cane fruits (22) included resistance tests of some of the trailing blackberries.

The most common dewberry or trailing blackberry native to California is *Rubus ursinus* Cham. & Schlecht. Several forms are recognized (7). The cultivated varieties Boysen, Logan, Mammoth and its thornless form known to the trade as Cory Thornless, Nectar, and Young are presumed to have been derived, at least in part, from *R. ursinus* (3). The Logan variety apparently resulted from a cross between the California wild (Aughinbaugh) blackberry and the Red Antwerp raspberry, or a similar European form; Mammoth, from a cross between the California wild blackberry and the eastern (Crandall) blackberry (18, 19); Young, from a cross between the

Phenomenal blackberry (an Aughinbaugh × red raspberry hybrid) and the Mayes dewberry (8); and Nectar, from seed of Young. The parentage of Boysen is not known, but it is similar to Young in most important botanical characteristics.

The varieties Boysen, Logan, Nectar, and Young are the most important commercially in California. The Himalaya (*R. proceras* Muell.), Oregon Evergreen (*R. laciniatus* Willd.), Crandall, Lawton (*R. allegheniensis* Porter), and Mammoth blackberries are of minor commercial importance.

The course of this disease was studied in commercial plantings and under more closely controlled conditions in an artificially infested field plot during the years 1946–1949 inclusive.

The terms primocane and floricane as defined by Bailey (3) are used in this paper. A shoot arising from the ground is, in its first year, a primocane; in its second year, in which it flowers and fruits, it is a floricane.

SYMPTOMS

Except in certain raspberry varieties, canes of infected brambles are not blue, as the earlier name blue-stem implied. Diseased canes may have bluish-brown streaks that appear to originate at the nodes and in severe cases cover a considerable portion of the cane.

Vascular discoloration is either absent in Verticillium-infected brambles of *R. ursinus* derivation, or if present is so faint as to be of little diagnostic value.

Symptoms on primocanes. Symptoms appear first on primocanes. In the coastal counties of central and southern California, affected primocanes, usually 1–2 ft. long, begin to wilt by the latter part of April. Some of the lowermost leaves only may wilt, but in severe cases the entire cane may droop.

Concurrently with the onset of wilting, yellowing of lower leaves begins, usually at the distal portions of leaflets, progressing inward between veins. Just one side of a leaf or only portions of leaflets may be involved. Yellowed areas invariably become brown and necrotic, giving the leaf a burned appearance (Fig. 2, A-C). Lowermost leaves show these effects most severely and commonly drop off early in the season, leaving the canes partially defoliated with a tuft of small green leaves at the tip (Fig. 1).

Environmental conditions greatly influence the rapidity with which yellowing and subsequent necrosis set in. Leaf necrosis may be greatly aggravated by an abrupt onset of hot, drying weather following a period of cool and overcast conditions. This combination of factors may cause leaves to die so rapidly that they often retain their green color after drying.

Rarely are all of the primocanes of a single plant diseased. Canes initiated during continuous warm weather, such as commonly occurs in both spring and summer in the interior valleys of California and during the summer months in the coastal counties, usually escape infection and grow normally while such weather persists. Some of these apparently healthy

canes may have symptoms—usually not severe—in the autumn. A growing period of 3 to 4 months with temperatures in the main above 80° F. enables a plant having severe symptoms in the spring and early summer to make an apparent recovery by producing a second crop of seemingly healthy primocanes. However, cultures made in November from 21 such canes yielded Verticillium in seven instances. This shows that infection may be well established in symptomless parts of the plant.

FIG. 1. Effects of Verticillium wilt on boysenberry in the spring: Wilting and drooping of primocanes and drying of their lowermost leaves are the conspicuous symptoms. (Photographed in May.)

Symptoms on floricanes. Certain of the apparently healthy canes produced during the warm part of the year invariably die during the following dormant season. These are assumed to have been infected earlier. Such canes die from the tips, usually to the ground. The widespread terminal killing of dormant canes, which for the past few years has been serious in California, should not be confused with Verticillium wilt. Its cause appears to be improper pruning and handling of canes, together with lack of water during the fall and winter months.

Floricanes that are not killed during the winter months usually produce leaves, set fruit, and mature in a normal manner. Under certain conditions,

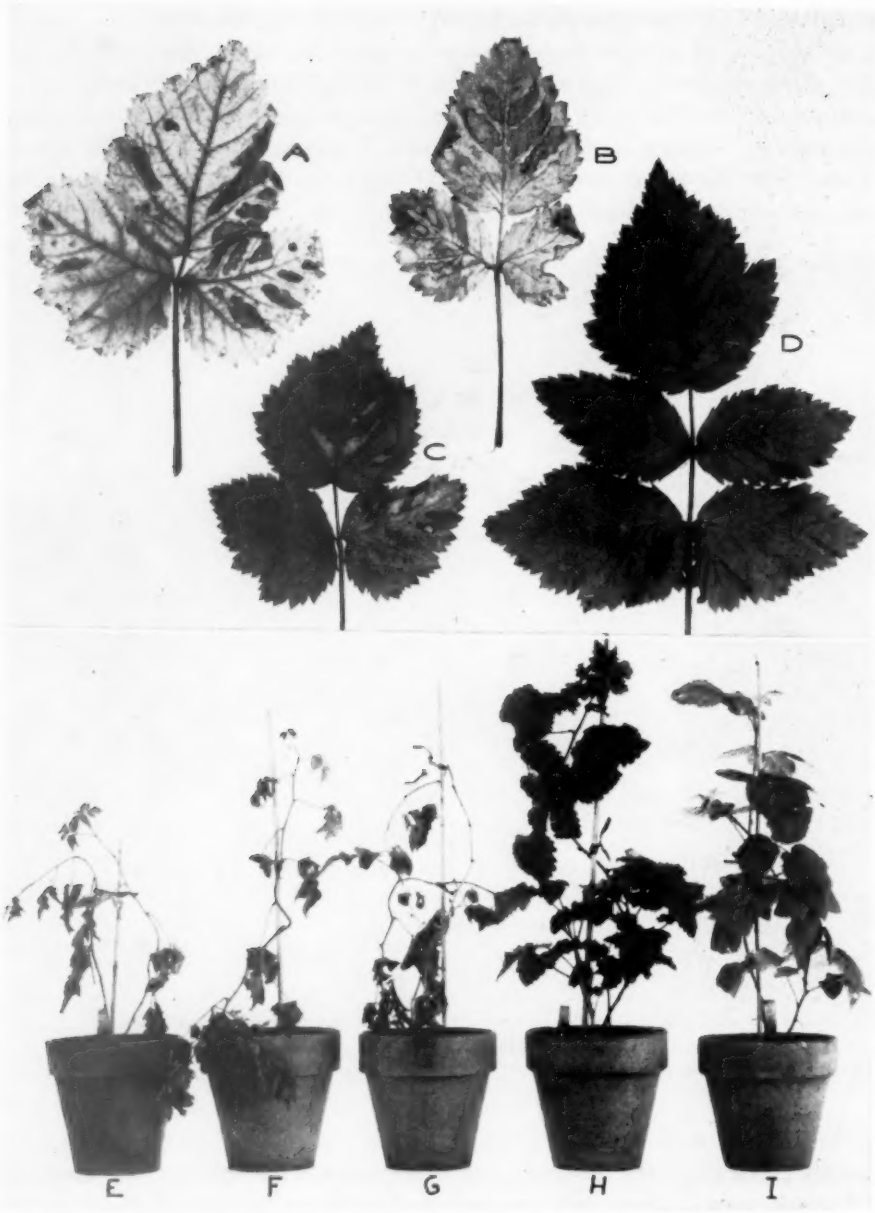

FIG. 2. Upper: A, B, C. Boysenberry leaves with symptoms of Verticillium wilt; D, healthy leaf. Extensive yellowing and interveinal necrosis on A and B. Some yellowing and necrosis in the lower right and terminal leaflets of C; the leaflet on the lower left is unaffected. Lower: E–H, seedlings of Boysen variety; E, F, G with severe symptoms 1 month following spray inoculation; H, resistant Boysen seedling. I. Resistant Logan seedling.

however, the entire floricane growth may collapse and dry at the time of fruiting. Again, an abrupt change from prolonged cool or overcast weather

to warm at the time the fruit is beginning to ripen appears to bring on this severe type of plant collapse, with resultant nearly total loss of the crop.

ETIOLOGY AND SUSCEPT RANGE

Verticillium albo-atrum Rke. & Bert. is the cause of Verticillium wilt of brambles. This fungus may be isolated readily from the vascular tissues of primocanes and floricanes having symptoms, and also from a percentage of the apparently healthy primocanes produced in the summer. Several isolates from diseased brambles (Boysen, Young, and Crandall varieties) from coastal and interior parts of California have with one exception been pathogenic for Bonny Best tomato in greenhouse inoculation tests. Likewise, several isolates from tomato have caused Verticillium wilt in seedlings of the Boysen and Young varieties.

Inoculation tests and field observations of commercial varieties and Rubus species have shown the following to be highly susceptible to Verticillium wilt: clones of the Pacific Coast trailing blackberry (*R. ursinus*) and its derivative commercial varieties Boysen, Young, and Nectar; the Crandall blackberry (derivative of *R. allegheniensis*); the thimbleberry (*R. parviflorus* Nutt.); the Cumberland Blackcap raspberry (derivative of *R. occidentalis* Linn.); and the Willamette, Lloyd George, Ranere, and Cuthbert red raspberry varieties (derivatives of *R. idaeus* Linn. or *R. strigosus* Michx.). The Cuthbert raspberry, though readily infected, displayed considerable tolerance. This agrees with work published earlier by Zeller (22).

Varieties and species that were highly tolerant or immune are: clones of the Pacific Coast trailing blackberry (*R. ursinus*) and its derivative commercial varieties Logan, Mammoth, and Cory Thornless (thornless Mammoth); the Himalaya (*R. proceras*), Oregon Evergreen (*R. laciniatus*), and Burbank thornless, known also as Sebastopol or Santa Rosa (*R. ulmifolius* var. *inermis*), blackberries; and a trailing species, possibly *R. procumbens* Muhl., from the southeastern United States. These results are in general agreement with those of Zeller (22) who earlier reported that the Himalaya and Oregon Evergreen blackberries were immune, and the Logan and Mammoth varieties comparatively resistant.

TESTS FOR RESISTANCE

Plants tested for resistance were inoculated by dipping the roots into a spore suspension of *V. albo-atrum* and planting in an infested field. During the first year the planting was intercropped with tomatoes. The plants were randomized in the field planting, and where possible at least six of each clone were tested. During the second year cultures were made from the crown tissues of one-half of the plants of each clone. Verticillium was recovered only from the varieties and species listed above as susceptible, with the addition of the Mammoth blackberry. At no time, however, did individuals of the Mammoth variety have symptoms, nor have symptoms been

observed in this variety in commercial plantings, even when planted in infested land where adjacent Boysen plants had severe symptoms.

It is significant that certain individuals of the Pacific Coast trailing blackberry are resistant and others susceptible to Verticillium wilt, and that among its derivative varieties, Logan and Mammoth are resistant, while Boysen, Nectar, and Young are susceptible. This was investigated further by testing the reaction to Verticillium of seedlings of some of the above varieties.

Tests with seedlings. Seedlings were grown to about 1 ft. in height. The bare roots were washed and dipped into a spore suspension at the time of potting into 6-in. pots. Thereafter, at the time of repotting to 7-in. and later to 8-in. pots, the roots were sprayed with a spore suspension without removing the soil. For the spray inoculation a paint gun operating at about 30 lb. air pressure per square inch was used. The survivors were washed free of soil, stored during the winter, and planted the following spring in an infested field plot after the roots were again dipped in a spore suspension. It should be pointed out that not all susceptible individuals were eliminated by a single inoculation, although the first inoculation killed by far the greatest percentage (Fig. 2, E–I).

The following numbers of seedlings have survived four inoculations such as were described, and also the first year in an infested field planting, without showing symptoms: 23 of 298 Thornless Young, 11 of 393 Young, 24 of 344 Boysen, 3 of 12 Nectar, 14 of 26 Pacific Coast trailing blackberry, and 71 of 71 Logan. Commercial Boysen and Young plants interplanted among the survivors had severe symptoms. In addition, small numbers of the F_1 progenies of the crosses Logan (resistant) × Lloyd George raspberry (susceptible) and Boysen (susceptible) × Himalaya (resistant) were all resistant. The Logan variety appears to breed true for Verticillium resistance. The Young, Nectar, and Boysen varieties (probably hexaploids) appear to segregate for resistance and susceptibility, as does the Pacific Coast trailing blackberry.

THE RELATION OF TOMATO TO INFESTATION OF LAND WITH THE WILT VERTICILLIUM

It is common knowledge among growers that land which has been cropped to tomato, potato, or cotton is, almost without exception, infested with the wilt Verticillium. The role tomato plays in the possible introduction of the fungus and subsequent intensification of the infestation was studied as follows: The percentage of plants infected with Verticillium wilt in 5 first-year tomato fields and 10 fields with a longer tomato history was determined by culturing 100 consecutive tomato plants of each field from a single row selected at random. Although symptoms were usually apparent, culturing was done to determine definitely the presence or absence of Verticillium infection. Cultures were made during or toward the end of the harvest period. Five first-year fields (not previously in tomatoes) gave 1,

1, 1, 9, and 12 per cent infected plants; six second-year fields, 18, 33, 36, 61, 74, and 75 per cent; third-, fourth-, fifth-, and tenth-year fields, 63, 77, 91, and 96 per cent, respectively. The first- and second-year fields had been field-seeded, with the exception of the first-year field, giving 12 per cent infected plants. This would for the most part preclude introduction of the fungus with transplants. In most of the fields having from 2 to 10 years of tomato history, cropping with tomatoes was not continuous. Barley, sugar beet, alfalfa, and bean were the common crops used in the rotations. Though inoculum blown with dust (21) or established in new land on weed hosts (16) is a more plausible explanation for the initial infestation of land than introduction with tomato (15), seed-bed infection excepted, two seasons of tomatoes cause an exceedingly rapid build-up of the infestation.

It might also be pointed out that Bandmann (4) in 1894 and Bewley and Buddin (6) in 1921 isolated *V. albo-atrum* from water supplies. It is, therefore, possible that land could become infested through the medium of irrigation water.

PREVENTIVE MEASURES

There is no economical soil treatment, chemical or other, known that will eliminate a Verticillium infestation from agricultural lands. Three- to 4-year crop rotations with nonsusceptible crops as alfalfa, clover, grain, and vetch are reported to be beneficial in some places (12, 17, 22), but little or no value from such rotations has been observed in California.

Land cropped previously to tomato, potato, or cotton, or on which the nightshade weed *Solanum nigrum* L. has been permitted to grow (16), should be avoided where planting of the varieties Boysen, Nectar, or Young is under consideration.

It also follows that propagation with the intent of sale should not be done in diseased plantings since rooted tips, though apparently healthy, and soil adhering to the roots may contain the fungus.

SUMMARY

Verticillium wilt is one of the most destructive diseases of bramble fruits in California. Heaviest losses occur in the coastal counties, particularly in years in which a prolonged cool or overcast growing season is followed by abrupt onset of hot weather.

The main symptoms are wilting and defoliation of primocanes, killing of floricanes at the time of fruit ripening with resultant loss of crop, and death of canes during the winter season.

The horticultural varieties of *R. ursinus* derivation, Boysen, Nectar, and Young, are susceptible, whereas Logan and Mammoth are highly resistant. The species *R. proceras, R. laciniatus, R. ulmifolius* var. *inermis,* and probably *R. procumbens* are resistant. Susceptible as well as resistant forms occur in *R. ursinus. Rubus parviflorus* and *R. allegheniensis* (Crandall variety) are susceptible.

Seedlings of the Boysen, Nectar, and Young varieties segregate for susceptibility and resistance. The Logan variety appears to breed true for resistance.

Tomato crops build up soil infestation with Verticillium. Tomato fields planted for the first time may show 1 to 12 per cent of infected plants, the second time 18 to 75 per cent, and the third to tenth time 75 to 96 per cent.

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INVESTIGATIONS OF MEADOW NEMATODES ATTACKING BOXWOOD, AND THE THERAPEUTIC VALUE OF SODIUM SELENATE AS A CONTROL[1]

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(Accepted for publication July 20, 1950)

The trend in the control of plant nematode diseases has been, in the past, primarily toward the application of soil fumigants (3, 16), use of noninfected plants in noninfested soil (7, 10), and crop rotation (7, 9, 10). These measures have been effective in increasing yields of annual crops and, in special cases, increasing yields of relatively quick-growing, woody, perennial fruit crops such as peaches. The only means of controlling nematode infection in most perennial plantings, however, is to destroy the plants and replant with resistant species or varieties in clean or fumigated soil.

Boxwood has been reported as being attacked by meadow nematodes, *Pratylenchus* spp. (6, 17, 19, 22, 26). Whereas plants in normal growth will form deep-green, compact foliage, symptoms of meadow nematode injury consist of a chlorosis and reddening of foliage and later defoliation. Examination of the dwarfed root system of meadow-nematode-infected plants reveals brownish-black lesions which eventually girdle the roots, causing the distal parts to decay. Plants in the early stages of infection send out lateral roots above the points of injury: these roots may also be attacked. Finally the plant forms a densely packed, shallow root system lacking deep-feeding roots. The lesions, at their junction with healthy root tissue, usually contain colonies of meadow nematodes (Fig. 1, A). These colonies are made up of males, females, larvae, and eggs, and usually are associated with saprophytic nematodes.

The organism regarded as causing this disease was originally referred to as *Pratylenchus pratensis* (de Man) Filipjev. Dr. G. Steiner, however, has found that several morphologically different forms are included in this species, and is currently revising the group.

Meadow nematodes measure from 300 to 700 μ in length. Their bodies are rather stout with the head end well set off, the tail end usually short and obtuse. The cuticle is noticeably annulated and is divided by the lateral fields, each consisting of four ridges and three grooves. The short buccal

[1] Scientific Article No. A251, Contribution No. 2186, of the Maryland Agricultural Experiment Station, Division of Plant Pathology, College Park, Maryland. Based on a thesis submitted to the Graduate School in partial fulfillment of the requirements for the Master of Science degree.

[2] The writer is indebted to Dr. Gotthold Steiner, Division of Nematology, Bureau of Plant Industry, Soils, and Agricultural Engineering, U. S. Department of Agriculture, Beltsville, Maryland, and Dr. Walter F. Jeffers, Department of Botany, University of Maryland, under whose joint direction this work was carried out.

This project was carried out under a fellowship sponsored by the Bartlett Tree Expert Co., Stamford, Connecticut, and in cooperation with the Bureau of Plant Industry, Soils, and Agricultural Engineering, Beltsville, Maryland.

stylet is sturdy and has well-formed basal knobs. In the body are a spherical-to-oblong medial esophageal bulb and enlarged esophageal glands which overlap the intestine. Both males and females exist in some species; in others only females have been found (20).

Selenium has been used successfully as an insecticide for a number of years (12, 14, 15). Its therapeutic potentialities as a nematocide have been investigated by Dimock (5), who controlled the foliar nematode of chrysanthemums, *Aphelenchoides ritzema-bosi* (Schwartz), by treating the soil with solutions of sodium selenate. He found that while 50 p.p.m. would prevent nematode infection of the plants, considerable selenium injury on the lower foliage as well as a slight stunting of the plant occurred. Skinner (17) applied sodium selenate to soil around meadow-nematode-infected roots of boxwoods, *Buxus sempervirens* var. *rotundifolia* Baill. and *B. microphylla* var. *japonica* (Muell. Arg.) Rehd. & Wils. He found that selenium treatment resulted in plants with glossy green foliage and long, straight feeding roots, as contrasted to the chlorotic foliage and bunched, broomlike roots of infected plants. Although the roots of treated plants were not free of meadow nematodes, infection was not extensive.

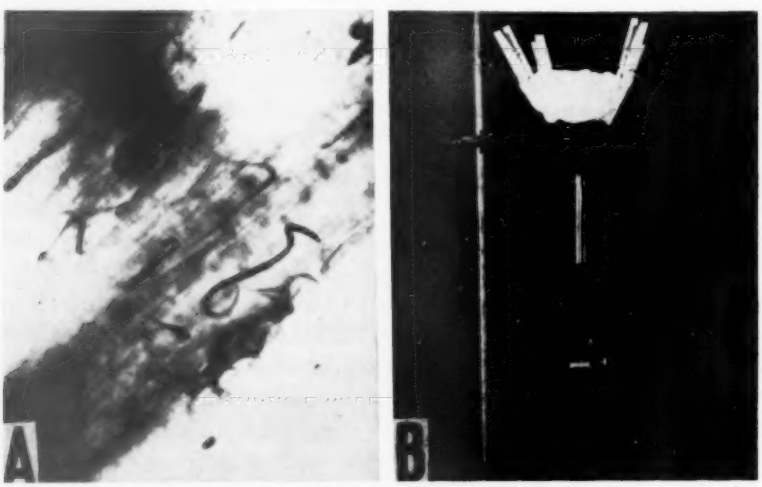

FIG. 1. A. Portion of an infected boxwood rootlet containing a colony of meadow nematodes stained with osmic acid and cleared in methyl salicylate. (×35.) B. Side view of the Baermann funnel.

The object of the research reported in this paper was to determine the efficacy of sodium selenate in reducing or eliminating populations of meadow nematodes infecting roots of boxwoods. It was felt that the effective utilization of chemotherapeutic methods would alleviate the nematode problem, at least for select woody ornamentals, by the partial or entire eradication of the pathogen involved.

In the following experiments sodium selenate, when used, was applied to boxwoods infected with meadow nematodes growing in a relatively non-seleniferous soil in an area with considerable variation in rainfall. The

chemical was supplied either dissolved in water or in powdered form as a soil amendment. All concentrations were based on parts of sodium selenate per million parts of soil unless otherwise designated.

OBSERVATIONS ON OVIPOSITION AND HATCHING

Information regarding the number of eggs deposited by female meadow nematodes and hatching of these eggs was needed to carry out some of the experiments. Details of the life history of these pathogens are still obscure, and reports by various workers are conflicting. Hastings (11) estimated the life cycle of the meadow nematode infecting oats in Canada to require about 54–65 days for completion, and suggested that the female nematode does not lay more than one egg per day. Gadd and Loos (8) estimated the life cycle of the nematode infecting tea in Ceylon to require 45–48 days, and stated that eggs may hatch in as short a time as 12 days *in vitro*.[3]

In an attempt to find some indication of the rate of oviposition and the time required for hatching, 12 gravid females were introduced into drops of water hanging from the top halves of small crystallizing dishes $1\frac{3}{4}$ in. in diameter and $\frac{3}{8}$ in. high. A shallow layer of water was placed in the bottom half of each dish, and the upper half containing the nematodes was replaced. These small moist chambers prevented evaporation of the hanging drops and permitted microscopic examination of the nematodes and eggs without separation of the two halves of the dish.

At 78° F., four of the gravid females laid 1 egg each, two laid 2 eggs each, one 3 eggs, one 6 eggs; four failed to lay eggs. One of the females laid four eggs in 1 day; two others laid two eggs each within the same time. Of the 17 eggs laid, 11 hatched; three of these required 5 days to hatch, and eight required 6 days. These results indicate that the meadow nematode infecting boxwood may have a shorter life cycle than the forms infecting some other hosts (8, 11).

TOXICITY OF SODIUM SELENATE TO MEADOW NEMATODES IN VITRO

The following concentrations of sodium selenate[4] were prepared: 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500, and 1,000 p.p.m. in tap water. Three active fourth-stage (preadult) or adult meadow nematodes obtained from infected boxwood roots were put into each of the solutions in miniature Syracuse watch glasses, and these were immediately covered to prevent excessive evaporation of water and change in concentration of selenium in the solutions. The watch glasses were stored in the laboratory at room temperature (80° F.).

During 6 days when the nemas were observed daily, none of the concentrations used had any noticeable effect on the nematodes. It was concluded

[3] Dr. Steiner has informed the writer that these forms of the meadow nematode differ from the forms infecting boxwood.

[4] The chemical used in all experiments was purchased from the Plant Products Corporation, Blue Point, New York. It was obtained in the anhydrous form colored with Ultramarine Blue No. 1 dye, and contained 41 per cent selenium by weight.

that sodium selenate at concentrations of 5 to 1,000 p.p.m. is not toxic to the meadow nematode when in contact with the integument of the animal. This experiment did not demonstrate the effect of ingested sodium selenate.

EFFECT OF SODIUM SELENATE IN SOLUTION ON ROOT-NEMATODE POPULATIONS OF 12-YEAR-OLD NURSERY-GROWN BOXWOODS

It was found, from previous experimentation, that 9-month-old dwarf English boxwoods, *Buxus sempervirens* var. *suffruticosa* L., would safely absorb selenium from concentrations of sodium selenate applied to the soil in solutions up to and including 100 p.p.m. Two months after treatment, when the roots of these plants were analyzed by a colorimetric method proposed by Trelease and DiSomma (25), the selenium concentration was as high as 78 p.p.m. Consequently an experiment was conducted to determine the effect of selenium on populations of nematodes in the roots of meadow-nematode-infected boxwoods growing in a nursery.

If selenium did not destroy all of the nematodes in the roots, a suitable method for evaluating the effect of treatments would be necessary. Cobb (4) and Stöckli (21) presented methods for estimating nema populations in the soil. Cobb used a method of decantation and also used various sized mesh screens to separate different species of nematodes from the soil and from each other. Stöckli counted the number of nematodes contained in a small amount of soil immersed in water. Neither of these methods was considered suitable for the purposes of this experiment. Reference was made, in Stöckli's work, to the investigations of H. Goffart in quantitative determinations of free-living nematodes.[5] According to Stöckli, Goffart immersed soil samples contained in a cheesecloth bag into a Baermann funnel filled with water. This apparatus (Fig. 1, B) consists of a glass funnel, to the end of which is attached a section of rubber tubing which is closed by a clamp (7). Once the soil samples were immersed, free-living soil nematodes began evacuating the samples and continued to do so for 10 days, the greatest evacuation occurring during the first 2 days.

Although Goffart's method applied only to free-living soil nematodes, it had long been a standard procedure to immerse finely cut nematized roots in water to induce the nematodes to evacuate the tissues. Here, as with soil, the majority of the nematodes within the roots seemed to evacuate during the first 2 days of immersion. Consequently it was theorized that Goffart's method for obtaining quantitative determinations of free-living nematodes in the soil would also be applicable for roots, and a procedure was devised for quantitatively assaying the degree of infection in nematized samples (23, 24).

Treatments were applied to 12-year-old plants of *Buxus sempervirens* var. *angustifolia* (Mill.) West and *B. sempervirens* var. *handsworthii* (K.

[5] Goffart, H. Methoden zur Prüfung von Pflanzen- und Vorrat-schutzmitteln. Reichsanstalt für Land- und Forst-wirtschaft 55: 155–164. 1937.

Koch) Dallimore[6] growing in an ornamental nursery on the grounds of the Plant Industry Station at Beltsville, Maryland, on August 13, 1947. These plants were growing in a gravelly, loamy sand with the following characteristics: hygroscopic moisture, 0.6 per cent; pH, 5.9 per cent; organic matter content (determined by loss on ignition), 3.2 per cent. Mechanical analysis (2): rocks (2 mm. or larger), 7.4 per cent; sand (1.0–0.05 mm.), 78.8 per cent; silt (0.05–0.005 mm.), 7.9 per cent; coarse clay (0.005–0.002 mm.), 0.55 per cent; fine clay (0.002–000 mm.), 5.35 per cent.

The boxwoods had symptoms of meadow-nematode injury varying from slight bronzing of the new growth to 75 per cent defoliation. Concentrations of 25, 50, and 100 p.p.m. of sodium selenate in 10 l. of tap water were prepared and applied to clear circular areas with a radius of approximately 2 ft. around the trunks of four replicate plants for each treatment. Controls received 10 l. of tap water.

Each plant was sampled 3 days prior to treatment and at weekly intervals for 1 month after treatment. A sample consisted of roots from opposite sides of the plant and about 1 ft. from the trunk and 3–6 in. below the soil surface. The roots were washed, blotted dry, and cut into small pieces with scissors. One gm. of the chopped roots was then immersed in water in a Baermann funnel for about 1½ days, after which 10 ml. were drawn off and the nematodes therein counted in a Syracuse watch glass marked to permit counting of nematodes in opposite areas representing a total of one-third of the area of the dish (Fig. 3, A). At each sampling, 10 gm. of the soil from around the sampled roots was also immersed in a Baermann funnel in order to observe the effect of the selenium on soil nematodes.

As indicated in figure 2, A, all treatments, except the controls, resulted in lowered nematode populations in the roots for the first week after treatment. The nematode populations of plants receiving 25 and 50 p.p.m., however, began to increase during the week of August 20–27 and continued until the week of September 3–10, at which time they again decreased. The nematodes in plants receiving 100 p.p.m. decreased from August 10 to August 27, after which they increased up to the time the experiment was terminated. Inspection of maximum and minimum temperature and daily precipitation charts for August 10 to September 10, 1947, yielded no apparent explanation of the results. A possible interpretation, however, may be that the selenium was toxic only to living nematodes and not to the nematode eggs and caused a sharp decrease in the number of nematodes in the 25- and 50-p.p.m. treatments; once the eggs hatched, however, the nematode populations increased. The 100-p.p.m. treatment may have had a longer duration of toxicity.

Analysis of variance of the data showed no significant difference between the treatments, due to the great variation of individual replicates as well as the fluctuating effects of selenium treatments. The mean nematode count

[6] Twig samples were kindly identified by Dr. H. T. Skinner, Morris Arboretum, Philadelphia, Pennsylvania.

per plant receiving the 25-p.p.m. treatment was 257.65 ± 82.96; for the 50-p.p.m. treatment it was 205.55 ± 63.36; for the 100-p.p.m. treatment, 258.20 ± 83.30; and for the controls, 216.25 ± 13.34. The standard error for the control mean was much less than the standard errors for the treatment means, indicating that application of selenium had an effect on nematode populations in boxwood roots.

A study of soil populations indicated that selenium decreased the number of nematodes in the soil surrounding boxwood roots without the great fluctuations evidenced in the root samples (Fig. 2, B). The mean nematode count for the soil surrounding roots of plants receiving the 25-p.p.m. treatment was 141.00 ± 22.33; for the 50-p.p.m. treatment it was 111.55 ± 27.62;

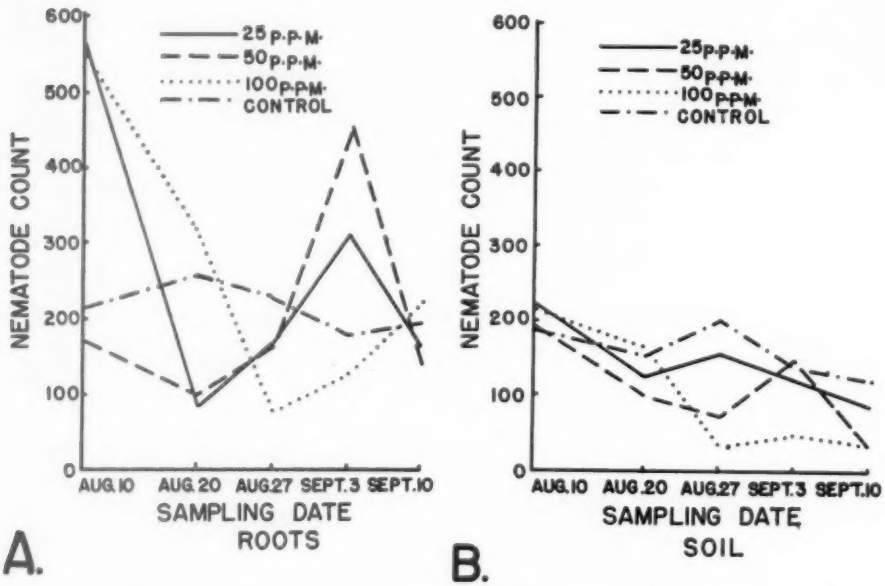

FIG. 2. Graphic representation of weekly nematode counts: (A) in roots, and (B) from soil surrounding roots, of boxwoods treated with sodium selenate in solution.

for the 100-p.p.m. treatment, 99.55 ± 37.04; and for the controls, 162.00 ± 14.41. Here also the differences between treatments were not significant because of variation between replicates, and, as with roots, the standard error of the mean of the controls was much less than the standard errors of the means of the selenium treatments.

The results of this experiment show that selenium temporarily reduced nematode populations in roots and surrounding soil. There were no clear-cut results, however, indicating which treatment was most satisfactory. The very striking variations of replicates within treatments suggested the possibility of differential absorption of selenium by individual plants, differences in the initial number of nematodes infecting the plants, and variations in meadow-nematode populations as a result of the life cycle.

EVALUATION OF THE BAERMANN FUNNEL TECHNIQUE

The previous experiment had been designed under the assumption that a modification of Goffart's quantitative method (21) would be applicable to meadow-nematode-infected boxwood roots. In view of the differences in nematode populations in replicate plants receiving the same treatment in the previous experiment, the validity of this method was investigated further. It was thought necessary to determine the length of time root samples should remain in Baermann funnels before the number of nematodes emerging would give an accurate estimate of the total nematode population in those roots.

Two-gram samples of meadow-nematode-infected roots from twenty-eight 13-year-old dwarf English boxwoods, some of which had received selenium treatments for another experiment, were immersed in Baermann funnels. Ten-ml. aliquots were drawn from each funnel three times weekly and put into the specially marked Syracuse watch glass (Fig. 3, A). The

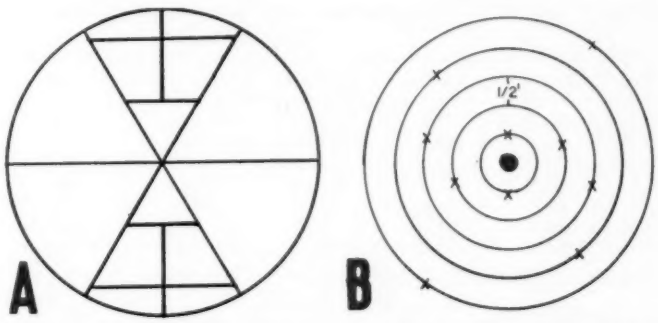

FIG. 3. A. Specially marked Syracuse watch glass used for counting one-third of the total number of nematodes present. B. Diagram illustrating a method employed for obtaining root samples from around the trunks of plants.

aliquot in each dish was then thoroughly agitated to insure an even distributions of nemas. Whereas various species of nematodes were always obtained from root samples, only meadow nematodes were counted in this and succeeding experiments since these were the parasites against which control measures were being directed, and since the possibility existed that their reaction to selenium may be different from that of other species. After a sample yielded no meadow nematodes for three successive samplings it was discarded. Examination of some discarded samples showed that evacuation of meadow nematodes was complete.

The majority of the samples yielded their nematodes within 4 to 5 weeks after immersion. The number of nematodes evacuated for each week up to and including the fifth week was counted, the percentage of nematodes evacuating each sample was then calculated, and the standard error for the mean of each group of weekly percentages computed. These data are in table 1.

After 1 day's immersion in Baermann funnels, only 19.2 ± 1.0 per cent of the total nematode population had evacuated the roots. Three weeks' immersion resulted in an evacuation of 95.6 ± 3.5 per cent, and immersion for a longer period did not increase the percentage evacuation appreciably. By the use of this 3-week immersion period, a valid indication of the total nematode population in the roots was obtained. On the basis of these results all succeeding root samples were immersed for 3 weeks, and aliquots were drawn from each Baermann funnel three times each week and accumulated in covered beakers. After each sampling a drop of commercial formalin was added to the contents of each beaker in order to inhibit bacterial decomposition of the nematodes, which would make identification of meadow nematodes difficult. At the end of the 3 weeks, a 5-ml. portion, representative of the composite aliquot, was put into the counting chamber (Fig. 3, A) and the meadow nematodes were counted (24).

TABLE 1.—*Percentages of the total number of meadow nematodes evacuating boxwood roots in Baermann funnels after various periods of time*

Sample number[a]	Time interval					
	1 day	1 week	2 weeks	3 weeks	4 weeks	5 weeks
1	20.9[b]	73.2	93.0	98.7	99.3	100.0
2	20.5	86.4	100.0	100.0	100.0	100.0
3	21.9	82.2	97.2	100.0	100.0	100.0
4	15.5	77.6	100.0	100.0	100.0	100.0
5	19.0	81.0	96.6	98.3	100.0	100.0
6	15.8	81.6	94.7	97.4	100.0	100.0
7	21.1	40.4	58.4	74.5	86.3	89.4
Mean ± Standard error	19.2 ± 1.0	74.6 ± 5.9	91.4 ± 5.6	95.6 ± 3.5	97.9 ± 1.9	98.5 ± 1.5

[a] Samples 1–6 were from selenized plants; sample 7 from untreated plants.
[b] Each percentage represents the mean of four replicates.

INVESTIGATIONS ON THE UNIFORMITY OF INFECTION

Since a 3-week immersion period gave a more reliable estimate of the total nematode population than a 1½-day immersion, an experiment was designed to measure the uniformity of infection of root systems of boxwoods attacked by meadow nematodes. Meadow nematodes are regarded as migratory parasites. When the source of food within a parasitized rootlet diminishes as a result of the prolonged feeding of these pathogens, they evacuate the rootlet. In view of this fact, only plants showing the earlier symptoms of the disease were used. It was thought that the nematode population in these plants would be at its maximum and that evacuation of the nematodes from the roots would not have begun.

Four 13-year-old boxwoods of the variety *angustifolia* having early symptoms of infection, slight bronzing or chlorosis but no defoliation, were selected at random. Two root samples were obtained from opposite sides of each plant about 6 in. below the soil surface and 6 in. from the trunk. Ad-

ditional samples were taken from each of four imaginary concentric circles spaced 6 in. from one another (Fig. 3, B). The opposite root samples were combined, giving a total of five composite samples per plant of which no two lay in the same radius from the trunk. They were washed, dried, and finely chopped, and 2-gm. samples of each were immersed in Baermann funnels. A cumulative count of the meadow nematodes emerging was made during a 3-week period of immersion. A standard error of the mean of 10 per cent or less was considered as the limit of accepted variability for the samples from each plant. Only the mean for plant No. 3 had a standard error of slightly more than 10 per cent (Table 2).

These results indicate that boxwoods growing under the same conditions and having approximately the same symptoms of early infection will sustain a uniform infection throughout the root systems.

TABLE 2.—*Comparison of nematode infection between samples of roots taken from 13-year-old boxwoods*

Plant number	Number[a] of meadow nematodes in each of 5 samples	Mean ± Standard error
1	35–47–29–36–42	37.80 ± 3.09
2	111–129–83–89–138	110.00 ± 9.61
3	24–41–46–27–33	34.20 ± 4.13
4	46–66–54–59–45	54.00 ± 3.96

[a] Nematodes in $\frac{1}{3}$ of counting dish.

EFFECT OF SODIUM SELENATE IN POWDERED FORM ON MEADOW NEMATODE POPULATIONS IN ROOTS OF 13-YEAR-OLD NURSERY-GROWN BOXWOODS

It is possible that sodium selenate applied in powdered form to the soil might have a more destructive effect against meadow nematodes than if applied in solution. Soil moisture would slowly dissolve the powdered compound and render it available to the plant over a long period of time.

To a group of twenty 13-year-old boxwoods of the varieties ***arborescens*** and *angustifolia* infected with *Pratylenchus*, treatments of 50, 75, and 100 p.p.m. of sodium selenate powder in 500 gm. of soil were spread evenly over a cleared circular area with a radius of $2\frac{1}{2}$ ft. from the trunk of each plant. Controls received an equal amount of soil. Each of the four treatments was applied to single plants randomized within five replicated blocks. Samples of roots from opposite sides of each plant were combined and analyzed 1, 3, and 5 months after treatment. Two gm. of these composite samples were immersed in Baermann funnels and nematodes were counted.

Sodium selenate as a powder seemed to be more effective than in solution (Table 3). Both 50- and 75-p.p.m. treatment means were significantly lower at the 1 per cent level than the control mean.

In table 3 it is indicated that samples obtained 1 month after treatment might give a satisfactory estimate of the efficiency of treatment, and also that the 75-p.p.m. concentration was most effective in decreasing meadow

nematode counts. Thus, in other experiments to determine the best soil area for treatment, this concentration of the powdered chemical in 500 gm. of soil was applied to boxwoods of the varieties *arborescens* and *angustifolia*, randomized within five replicate blocks. Cleared areas around the trunk of each plant were treated: areas 1 ft. in radius, areas 2½ ft. in radius, and areas between two concentric circles, 1 and 2 ft. from the trunk.

Root samples obtained 1 month after treatment had a mean nematode count of 22.0 for the treatment applied to an area with a 1-ft. radius, 25.8 for the treatment applied to an area with a 2½-ft. radius, 31.2 for the treatment applied between two concentric circles, and 54.0 for the controls.

TABLE 3.—*The effect of sodium selenate in powdered form on meadow nematode populations in roots of nursery-grown boxwoods*

Sodium selenate (p.p.m.)	Replication	Number of meadow nematodes observed			Mean[a]
		Sampling interval			
		1 mo.	3 mo.	5 mo.	
50	1	27	30	72	22.6**
	2	3	8	26	
	3	40	28	28	
	4	41	7	80	
	5	6	2	1	
75	1	4	1	30	15.1**
	2	1	3	16	
	3	59	20	37	
	4	4	12	6	
	5	3	29	1	
100	1	7	36	33	30.3
	2	7	6	30	
	3	43	36	71	
	4	38	49	27	
	5	7	56	9	
Control	1	33	3	34	40.5
	2	78	38	41	
	3	60	91	95	
	4	12	29	52	
	5	4	34	3	

[a] L.S.D. at 5 per cent level, 12.3; at 1 per cent level (**), 16.6.

These counts were not statistically significant because of the great variation among replicates of the same treatments despite the apparent reductions in populations in roots of sodium-selenate-treated plants in comparison to the controls.

EFFECT OF HOT-WATER-SELENIUM THERAPY ON MEADOW-NEMATODE POPULATIONS IN ROOTS OF BOXWOODS

Various meadow-nematode-infected plants have been treated successfully with hot water. Bally and Reydon (1) found that immersion of infected coffee plants at 49.5° C. for 10 min. achieved complete control of the parasites, and Mayne (13) found that only 75 per cent of treated coffee

plants would survive 15-min. immersion at 45° C. and 20-min. immersion at 41° C. Hastings (11) reported that a 10-min. immersion of infected oat roots at 120° F. (48.89° C.) completely destroyed the meadow nematodes contained within, and Gadd and Loos (9) controlled meadow nematodes in roots of tea plants with hot-water treatment.

Several thermal treatments of 6-month-old meadow-nematode-infected dwarf English boxwoods in active growth were carried out at 105°, 110°, and 115° F. for 30 and 60 min., and at 120° F. for 15 and 30 min. Five plants were treated in tap water and five plants were treated in a solution of 200 p.p.m. of sodium selenate (85 p.p.m. of selenium) at each of the temperatures and times of immersion. Controls consisted of five plants which were immersed in tap water and five plants which were immersed in 200 p.p.m. of sodium selenate solution for 60 min. at approximately 65° F.

Approximately 3 months after treatment, all plants were harvested over a period of 2 weeks. The root system of each plant was first evaluated as to the degree of visible infection by means of an adaptation of the root-knot index proposed by Smith and Taylor (18). The entire root system was then chopped fine and immersed in Baermann funnels, and on the following day a 10-ml. sample was drawn from each. The number of meadow nematodes present in each sample was rated as many, few, or none.

Thermal treatment, in selenium solution, did not completely destroy the nematode populations without being lethal to some plants in that particular treatment, with the exception of the 110° F., 60-min. treatment (Table 4). Plants, as well as nematodes, seemed to withstand a temperature of 115° F. much better in selenium solution than in water. Results indicate that, for selenium solution, treatment at temperatures between 115° F. and 120° F. for 15 to 60 min. might result in good control of meadow nematodes, while similar results could be obtained in water at 115° F. with immersion times from 30 to 60 min. Treatment in selenium solution for 60 min. at 110° F. resulted in complete elimination of the pathogens without injury to the plants, but the fact that similar control of nematodes was not obtained at higher temperatures suggests that these results may have been due to chance and that the need for further study at these temperatures is indicated.

DISCUSSION

In investigating certain details of the life history of meadow nematodes infecting boxwoods, it was found that female nematodes laid more eggs, and that these eggs required a shorter time for hatching, than has been reported by certain workers for meadow nematodes infecting other plants (8, 11). Dr. T. W. Graham[7] has maintained cultures of meadow nematodes on corn roots. In the course of the work reported in this paper, attempts to maintain meadow nematodes from boxwood roots on corn roots were un-

[7] Tobacco root disease studies (unpublished data), Pee Dee Exp. Sta., Florence, S. C., 1947.

successful. It is evident that the monosexual form of *Pratylenchus* infecting corn and the bisexual form of the nematode infecting boxwood possess different feeding requirements. It is also possible that the meadow nematodes infecting boxwood may differ from the forms infecting other perennial plants.

It was found in the course of this work that boxwood roots will absorb and retain selenium for at least 2 months, and that some of the meadow nematodes in the roots are killed by selenium treatment. It is assumed that ingestion of selenium in ionized form may cause death of the nematodes;

TABLE 4.—*Effect of hot-water-selenium therapy on root infection index and relative numbers of meadow nematodes in boxwood roots 3 months after treatment*

Temperature and duration of treatment	Root infection index[a] after		Relative number of nematodes[b] after	
	Water treatment	Selenium treatment	Water treatment	Selenium treatment
65° F., control	4–3–3–2–3	2–3–3–4–4	Many	Many
105° F., 30 min.	2–3–3–2–3	1–2–2–3–2	Many	Many
60 min.	2–2–2–1–2	3–3–3–3–2	Many	Many
110° F., 30 min.	0–1–2–1–2	2–2–3–2–2	None, 1, 3; few, 4, 5; many, 2	Many
60 min.	1–1–1–1–1	1–2–2–2–2	None, 1, 4; few, 2, 3, 5	None
115° F., 30 min.	1–1–1–1–1	1–1–1–1–1	None, 1, 3, 4; few, 2, 5	Few, 1, 2, 3, 4; many, 5
60 min.	0–1–D–D–D	1–1–1–1–1	None	None, 1, 2; few, 4, 5; many, 3
120° F., 15 min.	2–2–D–2–1	2–1–2–1–D	None, 1, 2, 4; few, 5	None
30 min.	D–D–D–1–2	D–2–D–D–D	None	None

[a] Root infection index given for five replicates: 0 indicates no infection; 1, 1 to 25 per cent infection; 2, 26 to 50 per cent; 3, 51 to 75 per cent; 4, 76 to 100 per cent; D, plants died as a result of treatment.

[b] When replicates varied in number of nematodes the replicate number follows the rating.

that selenium may combine with another substance in the plant tissues to form the toxic principle; or that another substance, toxic to the nematode, may be activated by the presence of selenium.

Details of the behavior of populations of *Pratylenchus* are as yet unknown. Knowledge of possible increases or decreases of the number of larval and adult nematodes is necessary. Lacking this information, it is possible that counts taken before treatment might be obtained when the population was numerically low, and that subsequent counts might be obtained after treatment when the population was numerically high. Such data would give an erroneous conception of the effectiveness of a treatment. If the fluctuations in nematode populations were known, root samples could

be obtained when the parasites were at a constant point on the population curve and this variable would be eliminated.

In experiments designed to control meadow nematodes in roots of boxwood, it is desirable to work with plants having a uniform nematode infection. This could be accomplished by growing plants in a uniformly infested soil. To insure the presence of sufficient inoculum in a potting soil, finely chopped, nematized roots would be thoroughly mixed with the soil and the plants grown in this mixture. A better method of insuring uniform infestation might involve the pouring of a suspension containing a standard number of meadow nematodes, in the same stage of growth, around the roots of each plant. However, one would have no assurance that each nematode was able to cause an infection. The inconclusiveness of these results with sodium selenate as a chemotherapeutic agent illustrates the complexity of this problem and indicates the necessity for further work.

SUMMARY

Boxwoods are subject to a root disease caused by meadow nematodes, *Pratylenchus* spp. Symptoms of the injury are chlorosis and bronzing of foliage, eventual defoliation, and necrosis of affected roots of the plant.

Some female nematodes, taken from boxwood roots and kept in the laboratory, may lay four eggs in 24 hr. and these eggs may hatch in a minimum of 5 days.

A 3-week immersion of root samples in Baermann funnels gave a valid indication of the total nematode population contained in those roots.

The root system of a plant with early symptoms of infection had a relatively uniform nematode infection at 6 in. below the soil surface.

Applications of 25, 50, and 100 p.p.m. of sodium selenate in solution to soil caused striking fluctuations of nematode populations in roots of 12-year-old boxwoods of the varieties *angustifolia* and *handsworthii* as contrasted to the controls.

Concentrations of 50, 75, and 100 p.p.m. of sodium selenate in powder form were applied to nursery-grown 13-year-old boxwoods of the varieties *angustifolia* and *arborescens*. Mean nematode counts from plants receiving 50 and 75 p.p.m. were significantly lower at the 1 per cent level than mean nematode counts from the controls. These results were not obtained in a subsequent experiment, however, when plants receiving 75 p.p.m. of sodium selenate powder applied to the soil in various ways were sampled 1 month after treatment.

Hot-water therapy, with the addition of 200 p.p.m. of sodium selenate, destroyed all of the meadow nematodes in the roots, but only at temperatures and times of immersion lethal to some of the replicate plants.

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RELATIVE BLIGHT RESISTANCE IN SPECIES AND HYBRIDS OF CASTANEA

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(Accepted for publication August 1, 1950)

Various species and hybrids of Castanea have been rated with respect to their relative resistance or susceptibility to the attack of *Endothia parasitica,* the fungus which induces chestnut blight. Ratings are based on thousands of test inoculations made during more than 30 years. Most of the inoculations were made with pure cultures of the fungus; in a few cases spore suspensions were used. Ratings for some of the rarer species and hybrids were based on personal observation of natural infections.

"Blight resistance" is only a relative term, and variations in disease reaction may occur, from complete immunity on the one hand to complete susceptibility on the other (6). Other factors being equal, the progress of chestnut blight is directly proportional to the rate of advance of the fungus in the host tissues. Thus, five reaction classes have been used in rating the chestnut species and hybrids: Class I, very resistant—no growth of fungus after insertion of inoculum. Class II, resistant—only slight growth; later, complete healing, callus forming around canker. Class III, somewhat susceptible—slow growth of fungus; callus may be formed but may be invaded later. Class IV, susceptible—fairly rapid growth of fungus, no callus formed. Class V, very susceptible—very rapid growth of fungus, no callus formed.

Twelve species and one variety were represented in our plantations by about 2,000 trees. Eight of the species have been given definite ratings. Four species and the variety have not yet been given definite ratings because they are represented by only a few individuals or because at present they are only in hybrid combinations. Most of these indeterminate cases are southern chinkapins and it is doubtful if they are hardy in Connecticut. The nomenclature is that of Standardized Plant Names, second edition.

Castanea mollissima Bl.—Chinese chestnut—Class I

Class I is the rating for the species, although much variation exists. Blight resistance is complicated by the comparative tenderness of the species, i.e., lack of resistance to cold (1, 2, 8). According to Reed (8), the Chinese chestnut requires "much the same climate as the peach." The fungus inducing the blight is a facultative saprophyte; it can and does live for long periods in dead wood or bark. When a branch, or part of it, is killed by extreme cold the fungus enters easily and, after establishing itself in dead tissues as a saprophyte, can more readily penetrate living tissue and adopt a parasitic existence. We have found, however, that trees more than 10 years old are more cold-resistant.

C. crenata Sieb. & Zucc.—Japanese chestnut—Class II

Great variation exists in different individuals and under different conditions. In this category is classed the form known as Japanese forest type as well as certain cultivated forms. It should be stated that there are 70 to 80 distinct varieties of the Japanese chestnut (7).

As with other chestnut species and hybrids, cultivation, pruning, spraying, and fertilizing are factors of the utmost importance in lessening blight susceptibility.

C. seguini Dode.—Seguin chestnut—Classes II, III, and IV

One accession of the species was rated in class II. It originated from nuts sent in 1935 by Mr. R. C. Ching of the Lu-Shan Arboretum and Botanic Garden, Han-Po-Kou, Lu-Shan, Kiu Kiang, China. It is a dwarf form, relatively hardy, and resistant to blight. Although the seguin chestnut was previously reported (4) not hardy in Connecticut, Mr. Ching in correspondence reported that it was abundant in the Lu-Shan Botanic Garden and survived the severe winter of 1930 when the temperature was as low as –15° F.

Another accession of the species was rated in classes III and IV. It was given us by the U. S. Department of Agriculture in 1929. It is not sufficiently hardy in Connecticut and dies back a little each winter. The blight fungus is apt to gain entry in the dead parts, but only one of the dozen originally received has been lost because of blight. None of the individuals is over 6 ft. high, and most are less than 3 ft. This accession differs in some characters from the 1935 accession. Dr. Donald Wyman of the Arnold Arboretum also reported in correspondence that the stock was not hardy in Massachusetts, for their plants died within 1 or 2 years and a plant grown from seed succumbed within 7 years.

C. pumila Mill.—Allegany chinkapin—Class III

No test inoculations have ever been made by us on this species. Although New Jersey is its northern native limit, it has so far proved fairly hardy in southern Connecticut with a little dieback at the ends of the branches in extremely cold winters. This, the common southern chinkapin, reputed to be fairly resistant to the blight, has nevertheless with us repeatedly had its stems blighted to the base. Because of its bushy habit, however, this is usually not serious.

C. ozarkensis Ashe—Ozark chinkapin—Classes III and IV

Two or three of our trees, now 15 ft. high (13 years old) have been attacked and killed by the blight. One, now attacked, is being inarched, the diseased tissue being cut out. This species is rated in class IV since the blight kills it so readily, but it might also claim a place in class III since a callus sometimes forms about the lesion.

C. henryi Rehd. & Wils.—Henry chinkapin—Class IV

This species, a native of central and western China, where trees occasionally are 90 ft. tall and 15 ft. in girth, is also known as the Chinese timber chinkapin, and is placed with the chinkapin group because it bears

only one small nut in each bur. The species has been said to be blight-resistant (3) but in our experience this is certainly not the case. At Hamden, Connecticut, all specimens except two have been repeatedly winter-killed and blighted, only to send up new shoots the next year. One of the two trees sent us in 1935 by the U. S. Department of Agriculture is in a fairly protected location and so far has not been winter-killed nor blighted.

C. sativa Mill.—European or Spanish chestnut—Class IV

This species, like *C. henryi,* is killed back each winter and becomes blighted. However, it usually comes up again from the root collar and makes a vigorous, rank growth, often 6 ft. in a year, only to die back and start over again the next year (7). In the fall of 1932 we received nuts of this species from Dr. E. Ulbrich of the Berlin-Dahlem Botanischer Garten, from Dr. B. P. G. Hochreutiner, from the Conservatoire Botanique de Genève, and from Dr. M. A. Guillaumin, Jardin de Plantes, Paris. We still have plants growing from these nuts, but in no case are the parts above ground more than 3 years old. However, the growth is so vigorous, the individual shoots so thick and sturdy, that it would seem a good stock to incorporate by breeding with a type of blight-resistant chestnut for future reforestation. We should appreciate receiving pollen of this species.

C. dentata Borkh.—American chestnut—Class V

This species is the most blight-susceptible of all chestnuts. The fungus, when it gets a start in young saplings, grows so rapidly that it is often impossible to save the tree either by cutting out the diseased bark or by inarching or by a combination of these methods. And yet there is within the species a marked variation as regards blight resistance. Figure 1, A, shows a group of resistant sprouts. The trees grow on the summit of a high rocky ridge in thin soil, so that the resistance is obviously genetic and not a result of favorable environmental influence. The principal sprout—the larger one near the center of the group showing a canker with a healing callus surrounding it—is evidently about 25 years old and came from an old stump, all that is left of a trunk which died above ground apparently about 1920. Some of the numerous small shoots surrounding the larger one are being inarched to prolong their life, for we are using this particular individual in our breeding work. Were this alone the sole representative of the American chestnut it could logically be assigned to class II or III.

C. alabamensis Ashe—Alabama chinkapin

We have one hybrid of this species with *C. seguini,* represented by one individual—not sufficient for a rating.

C. alnifolia Nutt., *C. ashei* Sud., *C. floridana* (Sang.) Ashe, and *C. floridana* var. *margaretta* Ashe endure Connecticut winters with difficulty, the hardiest being *C. alnifolia.* Thus, their behavior with respect to the blight is complicated by their tenderness.

HYBRIDS

Since we began breeding the chestnut in 1930 we have harvested in all up to and including 1949, 6982 hybrid nuts or an average of about 344 nuts per year. In 1949 the total harvest was 444. These nuts were all the result of controlled pollinations. Many hybrids become blighted during the early years of their existence. If they are F_2's they are promptly removed, but if F_1's of good parentage they are kept alive and vigorous by the inarching method, for further breeding. One of our plantations was begun in the years 1937–1940, with 600 hybrids. Now, as a result of culling out the undesirables, 91 remain, mostly of fine stock. Altogether we now have about 1000 hybrids, mostly in good condition. This includes about 150 at the Redding Ridge, Connecticut, plantation; 75 at the plantation of the White Memorial Foundation at Litchfield, Connecticut; about 200 in the six U. S. Department of Agriculture plantations; and the remainder at our Sleeping Giant Plantation at Hamden, Connecticut, and the Connecticut Agricultural Experiment Station Farm at Mount Carmel, Connecticut.

Our breeding records indicate that blight resistance is definitely a character, or probably a combination of characters, which, in the inheritance, follow the Mendelian pattern. But the original parents of wild species of chestnut, e.g. Japanese, Chinese, or American trees, are evidently heterozygous, a condition which results in lack of uniformity in the F_1 generation (5). The situation is further complicated by the fact that blight resistance is obviously not controlled by one gene only. Furthermore, a linkage with other characters may be a prerequisite to its appearance.

Since the F_1 generation is not uniform, the F_2 is even more heterogeneous. We have obtained several individuals of this F_2 generation and also of back crosses of the F_1 with resistant parents, which are evidently of the desired timber type. Unfortunately the chestnut is self-sterile, so that we cannot establish a pure line by inbreeding. Continued interbreeding and roguing would be a long process. The necessity therefore arises of starting clones of these desirable types. Since we have not yet been able to root cuttings, these clones, using scions from the desirable types, are at present being supported on resistant Chinese seedling stocks.

In order to shorten the hybrid names we have adopted the following abbreviations: A—American chestnut, *C. dentata;* J—Japanese chestnut, *C. crenata;* C—Chinese chestnut, *C. mollissima;* seg.—Seguin chestnut, *C. seguini;* S_8—Vanfleet hybrid, *C. crenata* × *C. pumila.*

The female parent is given first. Thus, JA means a Japanese-American hybrid in which a Japanese was the female parent, an American the male; JA × JA means that a Japanese-American hybrid used as the female has been crossed with another Japanese-American hybrid used as the male. It should be stated that the S_8 stock is derived from open pollinations of S_8 at Washington, D. C. Therefore, the male parent which enters into the formation of this S_8 progeny is unknown.

FIG. 1. A. Blight-resistant American chestnut, *Castanea dentata*. Roadside, Roxbury, Conn. Height of largest shoot, 26 ft.; circumference at base, 26 in. Note healed canker about 2 ft. up and others, higher. Inarching shows on large shoot left of center, above. B. Chestnut, C × JA (253–37). Sleeping Giant Plantation, Hamden, Conn. Class I as regards blight resistance, 11 years old, 21 ft. tall. One of the best of a group which show great promise for reforestation stock. (Photographed by L. Buhle, (A) Sept. 17, 1949, and (B) Sept. 18, 1948. Courtesy Brooklyn Botanic Garden.)

Among the JA hybrids few individuals were in the resistant class II, but among the hybrids obtained when JA was again crossed with J, many individuals were resistant. Among hybrids from the double cross JA × JA

a few trees were resistant and their resistance depended on the segregation for this character during the breeding (5).

More than 100 of the C × JA hybrids are in the plantations, ranging in age from 1 to 12 years. Some are excellent specimens from the forester's viewpoint (Fig. 1, B). As would be expected from the genetic situation, a few individuals are susceptible to blight, but generally the native resistance of the Chinese parent has combined with that of the Japanese sufficiently to overcome the susceptibility of the American parent. The Chinese stock shows marked dominance in this combination. Some other hybrids for which the Chinese parent was used are too young for blight reaction rating.

TABLE 1.—*Relative blight resistance of chestnut hybrids*

Hybrid	Blight resistance class	Hybrid	Blight resistance class
JA	II, III	C × seg.	I, II
JA × J	II, III	(C × seg.) × J	I, II, III
J × JA	II, III		
JA × A	IV	S_s	III, IV
JA × (JA × A)	III, IV	$S_s \times S_s$	III
JA × JA	II, III, IV	$S_s \times A$	III, IV
(JA × J) × (JA × J)	II, III, IV	$A \times S_s$	III, IV
		$S_s \times J$	II, III
CA	II, III	$S_s \times C$	II, III
AC	II, III	$(A \times S_s) \times C$	II, III
C × JA	I, II, III		

Hybrids from the C × seg. cross were in classes I and II with respect to blight resistance. The seguin parent was the U. S. Department of Agriculture stock of the species which is not hardy but seldom succumbs entirely to the blight fungus that often infects winter-killed shoots. Four of the hybrids have grown on our plantations since 1934 and three of the four have shown no signs of blight. They are prolific and bear small and sweet nuts, resembling the seguin parent in these characters. The fourth hybrid obviously is a different genotype. Although 14 years old, it never bloomed until 1949. It is moderately susceptible to blight, and continued inarching of basal shoots has been necessary to keep it in good health.

Castanea neglecta is a natural hybrid of *C. dentata* and *C. pumila*. Pollen of *C. neglecta* was sent us in 1940 by Mr. J. C. McDaniel from Alabama. It was used to pollinate the S_s material and several hybrids were obtained. They appear promising but are too young for blight reaction rating.

SUMMARY

In this paper we have evaluated for the various world species of Castanea (omitting some American chinkapin forms) and for many important hybrids their relative resistance to the attack of the fungus,

Endothia parasitica, which induces the disease known as chestnut blight. The evaluation was based on numerous inoculations from pure cultures of the fungus, and on personal observations over a period of more than 30 years.

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BACTERIAL FRUITLET BROWN ROT OF MEXICAN PINEAPPLE[1]

M. A. SMITH AND G. B. RAMSEY[2]

(Accepted for publication August 8, 1950)

In July 1949 a number of pineapples were received in this laboratory by air express from Mexico. Externally the fruit appeared sound but when cut open a brown mottling of the fruitlets was observed (Fig. 1). The decay was moderately firm. Examination of affected fruitlets showed that they were filled with masses of bacteria. When tissue plantings were made on potato-dextrose agar, yellow bacteria appeared around them within 3 to 4 days. After 7 days the organism had assumed a primuline yellow color.

A review of the literature reveals but few references to fruitlet brown rot of pineapple. One of the earliest is that of Tryon who reported a fruitlet core rot of pineapples from Queensland.[3] Barker in 1926[4] described a disease of pineapples in Haiti that affected 50 to 75 per cent of Smooth Cayenne fruits. A yellow organism that he isolated from diseased fruits proved pathogenic but he did not describe it. Serrano in 1924 observed a disease of pineapples in the Philippines similar to that described by Barker. He found that the disease was difficult to diagnose when fruits were observed from the exterior. In cut fruits the brown to bone-brown discoloration was often limited to the placental area of one or more fruitlets, although frequently the entire fruitlet was involved. All infected tissue was more or less filled with bacterial masses. Diseased tissue when cultured yielded a yellow bacterium which proved to be pathogenic. After a detailed study of the causal organism, Serrano[5] designated it as *Erwinia ananas*.

The disease of the Mexican pineapples received in this laboratory resembled that described by Serrano on Philippine pineapples. This report gives the results of studies of the cultural characteristics, biochemical reactions, and carbon metabolism of six isolates from diseased Mexican pineapples and records the results obtained in pathogenicity tests.

MORPHOLOGY

The organism is a short rod with rounded ends occurring singly or in pairs, but occasionally in chains. It is motile by peritrichiate flagella.

[1] Investigations conducted by the Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, in cooperation with the Department of Botany at the University of Chicago.

[2] Pathologist and Senior Pathologist, respectively, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture.

[3] Tryon, H. Fruitlet core rot of pineapple. Queensland Agr. Jour. **13**: 458–467. 1898.

[4] Barker, H. D. Fruitlet black rot disease of pineapple. Phytopath. **16**: 359–363. 1926.

[5] Serrano, F. B. Bacterial fruitlet brown-rot of pineapple in the Philippines. Philipp. Jour. Sci. **36**: 271–305. 1928.

Capsules were demonstrated. It is Gram-negative, is not acid-fast, and stains readily with either crystal violet or carbol fuchsin.

CULTURAL CHARACTERISTICS

Growth of the organism on potato-dextrose agar slants is at first straw yellow, becoming primuline yellow after 72–90 hr. On potato-dextrose agar dilution plates, after 30-hr. growth at 25° C. the surface colonies are approximately 4 mm. in diameter, circular, convex, dense, moist, straw yellow, and entire. On beef-extract agar slants the growth is at first straw yellow

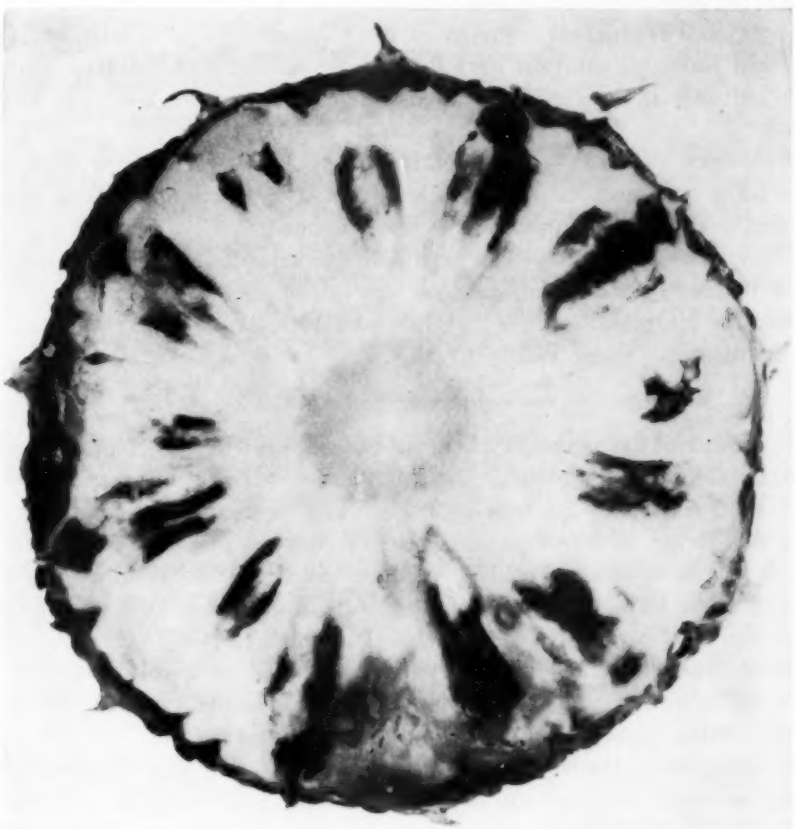

FIG. 1. Bacterial fruitlet brown rot of Mexican pineapple.

and raised, later becoming primuline yellow. Growth in nutrient broth becomes turbid after 22 hr., followed by the development of a fragile pellicle. After 10 days a yellow pigment is evident in the cultures. The purple color of brom-cresol-purple milk faded after 3 days and completely disappeared after 6 days. The medium was acid after 5 days, and coagulated. Growth of the organism in plain gelatin stabs is evident after 6 days. Growth is at first crateriform, later stratiform. Liquefaction is complete after 12 days.

BIOCHEMICAL REACTIONS

Relation to free oxygen.—When grown in Smith fermentation tubes, growth is evident first in the open arms and later progresses to the domes, indicating that the organism is a facultative anaerobe. No gas is formed.

Nitrate reduction.—In 36 hr. nitrates were reduced to nitrites, as shown by the red color produced when cultures were treated with alpha-naphthylamine-sulfanilic acid.

Indole production.—The various isolates were grown in Bacto-tryptophane broth and were tested for indole production at the end of 7 and 14 days. No positive reaction was obtained with any of the isolates.

Ammonia production.—Strips of filter paper saturated with a freshly prepared Nessler's solution were hung over 36- and 48-hr.-old beef-extract broth cultures of the various isolates. A positive test for ammonia was obtained.

Hydrogen sulphide.—Strips of lead-acetate paper failed to turn black when hung over beef-extract broth cultures of the various isolates, indicating that no hydrogen sulphide was being formed.

Hydrolysis of starch.—The organism growing in beef-extract broth to which 0.2 per cent of soluble starch was added was tested by the Eckford method for hydrolysis on the 2nd, 6th, and 10th days. The results showed that no diastatic action had occurred.

CARBON METABOLISM

In studies of carbon metabolism six isolates were used. Sugars, alcohols, and glucosides were added to a synthetic medium as recommended by the Society of American Bacteriologists.[6] The fermenting action of the bacteria was tested in fermentation tubes to which 1 per cent of the various sugars, alcohols, and glucosides had been added. Final results were noted after 21 days. The following are the results of the tests: Acid was produced in xylose, dextrose, fructose, galactose, mannose, lactose, sucrose, maltose, trehalose, melibiose, cellobiose, levulose, raffinose, glycerol, mannitol, sorbitol, and arbutin. Alkali was produced in rhamnose, levulose, melizitose, starch, inulin, glycogen, dulcitol, inositol, and erythrytol.

The results of the morphological, biochemical, and physiological studies of the bacterial isolates from Mexican pineapples agree in all essential details with those obtained by Serrano in his study of bacterial fruitlet brown rot of pineapples in the Philippines.

INOCULATIONS

In inoculation experiments it was necessary to use the Spanish Red, a variety grown in Cuba, because Mexican varieties were unavailable. Six

[6] Society of American Bacteriologists, Committee on Bacteriological Technique. Manual of methods for pure culture study of bacteria. Leaflet 2, Preparation of media. 9th Ed. 1944. Leaflet 4, Staining methods. 9th Ed. 1946. Leaflet 5. Routine tests for the descriptive chart. Morphological and biochemical. 11th Ed. 1949. Leaflet 6, Further biochemical methods. 9th Ed. 1942.

pineapples were used in the experiment. Five fruitlets on one side of each fruit were first punctured with a sterilized needle, after which a 7-day-old culture of the yellow organism originally isolated from diseased Mexican fruits was introduced by means of a sterilized needle into each of the punctures. Controls consisted of punctures made with a sterilized needle into fruitlets on the opposite side of each pineapple. A sixth pineapple was inoculated by introducing a 5-day-old broth culture of the organism into each of three holes made to a depth of 25 mm. with a sterilized cork borer having a diameter of 5 mm. Three similar holes made into fruitlets on the opposite side served as controls. After inoculation, all punctures and holes were covered with paraffin. The fruits were left in the laboratory at 23°–25° C. for 17 days; then cross-section cuts were made so that both inoculated and control punctures were revealed.

Bacterial infection had occurred in some of the fruitlets in each of the inoculated pineapples. Other fruitlets bore infections of *Penicillium* or *Fusarium* spp.; some fruitlets were so heavily invaded that accurate readings were not possible. The fungus contaminations were found in punctures into which the inoculum had been inserted, as well as in the control punctures. A number of bacterial reisolates were made, however, from inoculated fruitlets and the organism was recovered in pure culture. Control punctures remained free of bacteria.

SUMMARY

Specimens of Mexican pineapples affected with a bacterial disease of fruitlets were received in this laboratory in August 1949. Isolations made from diseased fruitlets yielded in all cases primuline yellow bacteria. Inoculation of Cuban pineapples with certain of these isolates proved successful. Detailed morphological, biochemical, and physiological studies were made of the organisms reisolated from inoculated fruit. A comparison of the results of such studies with those obtained by Serrano indicates that the organism isolated in this laboratory is *Erwinia ananas* Serrano.

So far as can be ascertained, this is the first report of the occurrence of this disease in North America.

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EFFECT OF TEMPERATURE AND RELATIVE HUMIDITY ON OCCURRENCE OF BLOSSOM BLIGHT OF STONE FRUITS[1]

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(Accepted for publication September 5, 1950)

Blossom blight of stone fruits (*Prunus* spp.) caused by *Sclerotinia fructicola* (Wint.) Rehm. is often destructive, wherever these fruits are grown (7, 13, 14, 26). It occurs sporadically in severe form on peach, cherry, and plum in the Northeastern United States and Eastern Canada (4, 6, 24). Direct loss to the current year's crop is caused by a heavy infection (4, 6), and further losses at harvest and in transit (24) may result from the fungus harbored in diseased blossoms and twig cankers.

The blossoms are present and are infected during April and May when environmental conditions are different from those prevailing at the time the ripening fruit becomes infected. The general acceptance of warm, humid weather as favorable for brown-rot infection of fruit may not apply to the blossom blight phase of the disease. Studies were undertaken, therefore, to determine the effect of temperature and relative humidity upon the incidence of disease by *S. fructicola* of blossoms of stone fruits. This paper presents the results of experimental studies from 1939 to 1942 concerning the effect of temperature and relative humidity upon the growth of the pathogen in culture and the incidence of infection in the blossoms. An abstract has been published (30).

SYMPTOMS

The first symptoms are brown lesions on petals, stamens, and pistil. During moist weather infected parts are soft and rotten; in a dry atmosphere they are discolored, dry, and brittle. From initial lesions on petals, anthers, or filaments, discolored areas rapidly extend to the calyx. Occasionally penetration of sepals occurs. Mycelium may permeate entirely the tissues of the petals and stamens 48 hr. after inoculation, and spread into the calyx cup. On the third day after inoculation the entire blossom may blight. From the calyx the pathogen spreads through the flower stem into the twig. Infection readily occurs on the stigma, but spread of the rot is comparatively slow in the pistil. It is not uncommon to observe complete rotting of stamens, calyx, and pedicel while the style is but partially brown.

[1] A revision of a thesis presented to the Faculty of the Graduate School of Cornell University, June, 1943, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

The author wishes to express appreciation to Dr. J. M. Hamilton for suggesting the present study and for his aid and advice during the course of the work, and for the use of the facilities of the Plant Pathology Department of the New York (Geneva) Agricultural Experiment Station. Thanks and credit are due to Dr. L. M. Massey whose aid and encouragement and whose criticism of the manuscript have been most helpful.

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In high relative humidity sporodochia of the fungus first appear on anthers and stigma. Later, fruiting structures are present on all floral parts. About 10 days after inoculation a gummy exudate may appear on the twig at the base of diseased blossoms. Cankers often form which may continue to enlarge and girdle the twig.

APPARATUS AND METHOD

The effects of temperature upon the initial infection of peach and cherry blossoms and the further development of disease were determined by inoculating flowers on potted trees, 2–3 years old, in large moist chambers similar to those described by Keitt and Jones (18). The temperature was regulated by spraying a mixture of hot and cold water on the inside roof and curtains of the chamber. Humidity was not controlled, but a saturated atmosphere was maintained at all temperatures. The buds were forced into blossom early in the spring by bringing the trees into a warm greenhouse. Trees used later were held outside until the blossoms reached the desired stage of development. The blossoms were inoculated by atomizing them with a spore suspension containing approximately 50,000 spores per ml., or by applying dry spores with a camel's-hair brush. In all cases, the inoculum was obtained from cultures grown on 2 per cent potato-dextrose agar. After a desired period in the saturated atmosphere of the chamber for germination and initial stages of infection, the trees were moved to the greenhouse unless otherwise stated. Records were taken of the development of disease on the individual blossoms daily or on every second day.

In the spring of 1942, three temperature- and humidity-controlled chambers were used, measuring 4 ft. 6 in. × 3 ft. 5 in. × 2 ft. 7 in., each capable of holding three or four short trees or many flasks containing floral shoots. These chambers were in a refrigerated room which had an average temperature of 11° C. They were made of 2 × 3-in. wood framing covered with ½ in. Celotex on three sides, top, and bottom, and with glass doors on the fourth side. A pan of water was placed on the floor of each cabinet and a false floor, which came to within 10 in. of the front, was built over it to hold the plant material. Air circulation was accomplished by a fan running continuously; the air was drawn from the bottom of the chamber over the water, under the false floor to the back, and behind a baffle to the fan at the top of the cabinet. The heating unit consisted of several incandescent lamps so situated that the air passed over the lamps at the top of the chamber. The temperature was controlled by a bimetallic helical coil thermoregulator operating a power relay which opened and closed the circuit to the heating source. The humidification was accomplished by the evaporation of water from the tray, which was heated by an immersion heater of 115-volt, 750-watt capacity. The relative humidity was controlled by means of a humidostat. The level of the water in the trays was held constant by float-valves. A continuous record was obtained by means of hygrothermographs.

THE PATHOGEN

The binomial, *Sclerotinia fructicola* (Wint.) Rehm., has been accepted for the pathogen causing brown rot since studies were reported by Roberts and Dunegan (25). Harrison (15) also has reviewed the taxonomy of the fungus and confirmed *S. fructicola*. Honey (17) has proposed the genus *Monilinia* for the monilioid species of *Sclerotinia*. The binomial *Monilinia fructicola* (Wint.) Honey has recently appeared in reports concerning studies with this organism (4, 10).

Hewitt and Leach (16) found both *S. fructicola* (Wint.) Rehm. and *S. laxa* (Ader. & Ruhl.) on stone fruits in California. The former was isolated most frequently from decayed fruit, the latter more frequently from blighted blossoms and twigs. These two species are readily identified by gross cultural characteristics. The development of *S. laxa* on sour cherry in Wisconsin was reported by Calavan and Keitt (3). *S. fructicola* is the only known brown-rot organism on peach east of the Rocky Mountains. Cultures used in these studies were fresh isolates from diseased peach blossoms; they were identified by their growth characteristics on potato-dextrose agar according to the description of Hewitt and Leach (16).

Temperature relationships. The effects of temperature on germination of conidia of *S. fructicola* have been studied by many authors (1, 8, 21, 29, 31). Nutrient substances are necessary for a uniform germination of conidia of this fungus (9, 19, 23, 32). Temperature studies of spore germination were made using potato-dextrose agar, peach petals, and glass slides coated with cellulose nitrate as suggested by Evans and Martin (11). By means of a platinum loop, conidia were taken from 7-day-old cultures to prepare a standardized spore suspension of 50,000 spores per ml. in tap water and in redistilled water. Three drops of a nutrient solution[2] (22) were added to 100 ml. of each spore suspension. Three drops of each spore suspension with nutrient were then placed on a glass slide. The slides were placed in Petri dishes which had been lined with moist filter paper. A single drop of the spore suspension in redistilled water without nutrient was placed on individual peach petals by means of a pipette. The petals were floated on 4 per cent sucrose solution in sterile Petri dishes. The conidial suspension, in redistilled water, was streaked across the surface of filtered media. After incubation at the desired temperatures, examinations were made at 1-hr. intervals. Petal samples were boiled in cotton blue in lactophenol for about 1 min. before examination under the microscope. Germination was considered to have occurred when over 50 per cent of the spores had germinated and the germ tubes had reached a length of from one to five times the spore width.

Conidia germinated over a wide range of temperature with an optimum range from 15° C. to 25° C. (Table 1). At low temperatures, 5° and 10°

[2] The liquid nutrient solution consisted of the following ingredients in the stated concentrations: M/4 dextrose, M/5 KNO_3, M/20 KH_2PO_4, M/100 $MgSO_4$, and a trace of $FePO_4$.

C., conidia germinated in the shortest length of time on peach petals. A promoting effect of nutrient substances and volatile materials from the suscept upon the germination of spores of *Botrytis cinerea* Pers. in the infection drop has been reported by Brown (2). A longer time was required for germination in tap water at pH 7.3 than in redistilled water with a pH of 6.0. Tilford (29), using mannite solution, reported a restricted temperature range for germination of conidia of *S. fructicola* at pH 7.2, while a pH reading of 6.0 was favorable at a wide range of temperature.

The effect of temperature on the radial growth rate of *S. fructicola* was studied. Each culture was grown on 18 cc. of 2 per cent potato-dextrose agar in a Petri dish. The Petri dishes were placed in the temperature-controlled refrigerators for about 12 hr. to come to the required temperature before planting. Five or more cultures were incubated at 5°, 10°, 15°, 20°,

TABLE 1.—*Relation of temperature to germination of conidia of Sclerotinia fructicola on different media*

Temperature of incubator	Time required for germination[a]			
	Potato-dextrose agar, pH 5.8–6.1	Tap water on glass slides,[b] pH 7.3	Redistilled water on glass slides,[b] pH 6.0	Peach petals
° *C.*	*Hours*	*Hours*	*Hours*	*Hours*
5	15.0–17.0	46.0–48.0	35.0	11.0–12.0
10	8.0– 8.0	24.0–26.0	18.0–19.0	6.0– 7.0
15	3.5– 4.0	9.0–10.0	6.0	3.0– 4.0
20	2.5– 3.0	6.0– 7.0	3.0– 3.5	2.5– 3.0
25	2.0– 2.5	5.0	3.0	2.0– 3.0
30	3.0– 3.5	.....	3.5– 4.0	3.0– 4.0
35		No germination	.....	No germination after 9 hr.

[a] Figures for each medium represent the results of three experiments.
[b] Nutrient solution added as given in text.

25°, 30°, and 35° C. Each Petri dish was planted with conidia taken from 7-day-old agar cultures with a loop 1.5 mm. in diameter. The diameter of each thallus was measured in millimeters at 24-hr. intervals. Growth was slow at 5° C. The optimum range of temperature for growth of *S. fructicola* was 20° to 25° C. Some growth occurred at 30° C., but the hyphal cells were short and abnormal. No growth occurred at 35° C. Ezekiel (12) reported the minimum, optimum, and maximum falling near 3°, 25°, and 33° C. respectively for five strains of *S. fructicola*.

The optimum temperature for sporulation was 25° C. Sporodochia were found in 10 days at 5° C., in 8 days at 10° C., in 3 days at 15° C., and in 2 days at 20° and 25° C. Sporulation at 30° C. was erratic.

Relative humidity studies. Investigators have shown that conidia of *S. fructicola* require precipitated moisture for germination on glass slides (5, 8, 20). In an experiment using salt solutions and water in Stender dishes to maintain relative humidities of from 93 to 100 per cent, dry spores on

glass slides failed to germinate at 20° C. after 24 hr. Germination occurred when a drop of water was placed on the spores.

In the study of the effect of relative humidity on germination of conidia, experiments were also conducted using floral parts of the peach as a substratum. Flowering shoots were used with cut ends in flasks of water. When a certain organ of the flower was to serve as the substratum, all the other floral organs were removed before the spores were applied. The conidia were dusted dry on the floral parts with a camel's-hair brush, or drops of a spore suspension were applied with a pipette. The flasks of peach shoots were then placed in humidity- and temperature-controlled cabinets. An optimum temperature was maintained at relative humidities of 80 and 96 per cent.

When dry spores were put on these floral parts, germination occurred only in a saturated atmosphere. At 96 per cent R. H., conidia did not germinate on the petals but the spores did germinate and penetrate the anthers and stigmas (Fig. 1). At 80 per cent R. H., germination occurred only on

FIG. 1. Photomicrographs of (left) germinating conidia in anther, and (right) the fungus on the stigma, of peach blossom. Sections made after 28 and 48 hr. respectively, at 96 per cent R. H.

the stigmas. Lack of liquid or water on the other organs prevented germination. When the inoculum consisted of a spore suspension, conidia germinated on all floral parts in a saturated atmosphere and at 96 per cent R. H. At 80 per cent R. H. germination occurred on petals and stigmas, but not on stamens.

Growth of mycelium. Since mycelial growth occurred over a wide range of temperature, 5° to 30° C., it is evident that temperature would rarely prevent its activity. Experiments were conducted, therefore, to demonstrate the effect of relative humidity on the rate of radial growth of *S. fructicola* in pure culture. Willison (33) reported growth-rate curves on potato-dextrose agar at 5° to 30° C. At the higher temperatures, drying of the agar retarded the growth rate, except at 30° C. where twice as much agar was used.

To establish equilibria between the solid culture media and relative hu-

midities, the method used by Shaw (27) was employed to determine those humidities that would permit growth of the fungus. Distilled water and sulfuric acid solutions with vapor pressures equivalent to the desired humidities were used. The acid solutions were prepared on the basis of the known relationship between their specific gravity and vapor pressure as given by Stevens (28) and by Wilson (34). The specific gravity of the acid solutions was determined by using a 25-cc. pycnometer. Briefly, the procedure in establishing equilibria between the acid solutions and the solid agar medium was as follows: Approximately 18 cc. of 2 per cent potato-dextrose agar were poured into the cover of each of a series of three sterilized Petri dishes. Small wooden blocks with holes made to hold the bottoms of Petri dishes were used. These blocks were washed with mercuric chloride 1–1000. After the Petri-dish bottoms were set in the blocks, about 30 cc. of the desired sulfuric acid solution were poured into each dish. The covers containing the solidified agar were then placed over the sulfuric acid solutions and were sealed to the blocks by sterile vaseline. The plates were then placed in an incubator held at 25° C. At intervals of approximately 3 days, the acid solutions were removed and fresh supplies were added. Equilibria were established between the acid solutions and the agar when there was no appreciable change in the specific gravities of the fresh acid.

Conidia were planted at the center of each plate. The plates were then placed in an incubator at 25° C. At the end of 2, 4, and 6 days, the diameters of the thalli were measured in each series of plates. Results of these studies show that maximum growth occurred on the media at the highest humidity equivalent (Fig. 2). In those plates at 100, 98.5, and 96 per cent R. H., growth was uninterrupted as indicated by the straight lines in the graph. At 93 per cent, growth was retarded after the fourth day; the agar had a leathery consistency. At 88.5 per cent, growth was checked after the second day, there being but a small increase in diameter after the fourth day. At 84 per cent R. H. there was but a slight increase in diameter after the second day. The fungus fruited on all of the plates.

EFFECT OF TEMPERATURE AND RELATIVE HUMIDITY ON DISEASE DEVELOPMENT

Open blossoms on 2–3-year-old potted peach trees were inoculated and placed in a saturated atmosphere under controlled temperatures for various lengths of time. Approximately 100 per cent R. H. was maintained at 20° C. or above, and slightly less than saturation at lower temperatures. Condensation water on the floral parts constituted an adequate supply of moisture for infection. The trees were moved to the greenhouse to observe the development of the disease. The procedure was changed in 1942 when, after certain intervals in saturated atmosphere, the trees were distributed to the greenhouse and to cabinets with controlled humidity and temperature.

The following approximate incubation periods were determined for blossoms held in a greenhouse environment on removal from saturated atmosphere: within 18 hr. at 10° C., 8–12 hr. at 15° C., 11 hr. at 20° C., and 5 hr. at

25° C. (Table 2). The blossoms were considered as completely blighted when the fungus had spread from infected floral organs through the calyx and peduncle of the flower. Initial symptoms of brown rot developed on floral parts which were in the saturated atmosphere for shorter periods of time. However, progress of the disease was checked on removal to the dryer atmosphere of the greenhouse. When the infected blossoms were subjected to a second incubation period, the disease spread and blossoms were completely blighted.

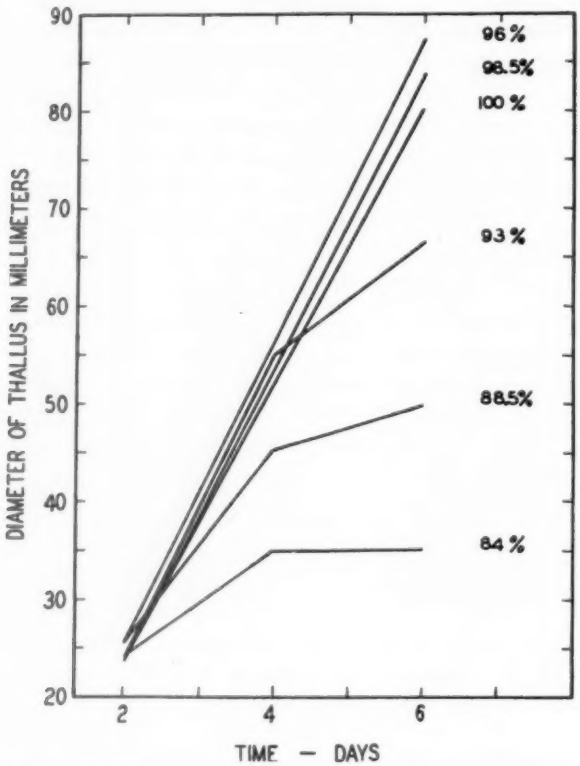

FIG. 2. Relation of relative humidity to rate of growth of mycelium of *Sclerotinia fructicola* in potato-dextrose agar at 25° C.

On removal of blossoms from a saturated atmosphere to controlled relative humidities and to the greenhouse environment, the highest percentage of blossom blight occurred at 90 per cent R. H. (Table 2). Blossoms subsequently held at 80 per cent R. H. and in the variable humidities of the greenhouse often failed to blight completely because of drying of the infected floral organs. Four hours at 80 per cent R. H. and 8 hr. at 90 per cent were required for the blossom parts to become dry after removal from saturated atmosphere. At relative humidities above 90 per cent, diseased areas in the petals remained moderately moist. At 80 per cent R. H., diseased spots were delimited, dry, and papery brown.

The blossoms held below 15° C. in the saturated atmosphere did not completely blight in any subsequent environment in less than 6 to 8 days. Blossoms held at 15° C. and above in the saturated atmosphere were destroyed in 80 per cent R. H. and in the greenhouse in 5 to 7 days, and in 90 per cent R. H. in 3 to 5 days.

In a series of experiments, records were taken to determine (a) the floral organs where infection occurred, (b) the liability to infection of blossoms in different stages of development, and (c) the period of susceptibility of blossoms and young fruit immediately after fertilization. These studies were made under controlled relative humidities.

TABLE 2.—*The relation of temperature to the development of blossom blight by Sclerotinia fructicola*

Temperature (° C.)	Hours held in saturated atmosphere	Percentage blossom blight following period held in saturated atmosphere[a]		
		Greenhouse	90 per cent R. H.	80 per cent R. H.
10	13.0	0.0	.....	.....
	16.0	1.8	.....	.....
	18.5	5.6	.....	.....
10–12	13.0	0.0	100.0	37.5
	16.0	0.0	.....	50.0
	19.0	7.1	100.0	100.0
	22.0	42.8	.....	100.0
15	8.0, 9.0	5.1	100.0	0.0
	11.0, 12.0	21.9	100.0	0.0
	14.0	31.7	100.0	56.0
20	6.0	2.6	100.0	0.0
	8.0	0.0	100.0	20.0
	11.0	13.3	100.0	100.0
25	5.0	5.3	.....	.....
	6.0	23.4	.....	.....
	7.0	48.0	.....	.....

[a] The range of temperature in the greenhouse was 7°–30° C.; the relative humidity varied from 40 to 100 per cent.
Temperatures in cabinets were held within range 15°–20° C.

Individual floral organs were inoculated with small droplets of a spore suspension. Every effort was made to prevent run-off from the desired infection court. The anthers were not removed from the stamens. The droplet of spores was placed on both the filaments and anthers. Parts of the blossom not to be inoculated were removed. In table 3, part A, are results of an experiment in which the floral organs were immediately placed in chambers with controlled humidity after inoculation. All floral parts are susceptible. In 88–90 per cent R. H., 3 days after inoculation, the fungus had spread into the calyx cup from infection of petal, sepal, and stamens. After penetration of the stigma, progress of the fungus down the style was comparatively slow, but the ovary and finally the entire blossom blighted. The fungus did not reach the calyx cup from petal infection in the majority of cases because the petals fell on removal to the humidity chambers. At

80 per cent R. H., neither stamens nor sepals became infected, but petals and pistils did. The diseased petals usually fell and the brown areas appeared dry. A small number of blossoms blighted from stigmatic invasion after 6 days. At 70 per cent R. H. invasion occurred by way of stigma only, and discoloration of style developed. Roberts and Dunegan (24) indicated that "all parts of the open blossoms may be attacked." In some of their experiments, typical blossom blight followed inoculation of the stigma with either conidia or ascospores. The flowers were held in high relative humidity under bell jars after inoculation with a spore suspension. In table 3, part

TABLE 3.—*Severity of infection by Sclerotinia fructicola of peach floral organs at different relative humidities, (A) immediately after inoculation and (B) following a period of wetness after inoculation*[a]

Relative humidity (per cent)	Temperature, ° C.	Number of blossoms inoculated	Floral organ inoculated	Severity of infection	
				After 3 days	Blossoms blighted after 6 days
					Per cent
A. 88–90	19	7	Petal	To calyx	28.6
		6	Sepal	do	100.0
		9	Stigma	To style	77.8
		6	Stamens	To calyx	100.0
80	15	7	Petal	To petal	0.0
		7	Sepal	None	0.0
		7	Stigma	To style	14.3
		6	Stamens	None	0.0
70	15	8	Petal	None	0.0
		4	Sepal	None	0.0
		7	Stigma	To style	0.0
		7	Stamens	None	0.0
B. 88–90	19	10	Petal	To flower stem	40.0
		4	Sepal	do	75.0
		13	Stigma	To style	69.2
		6	Stamens	To flower stem	100.0
80	15	10	Petal	To petal	10.0
		5	Sepal	To sepal	0.0
		12	Stigma	To style	41.6
		10	Stamens	To stamens	100.0
70	15	8	Petal	To petal	0.0
		6	Stigma	To style	16.7
		8	Stamens	To stamens	62.5

[a] In saturated atmosphere 15 hr. at 15° C.

B, are presented data on the severity of infection of floral parts in different relative humidities following inoculation and incubation for 15 hr. at 15°–19° C. Discoloration was present on all inoculated organs on the second day after inoculation at 88–90 per cent R. H. Infection had spread into the calyx from petals, stamens, and sepals on the third day. Blossoms blighted from pistil infection after 6 days. In 80 per cent R. H. the disease progressed slowly in the petals and sepals. After 6 days, infection spread from stigma and stamens through calyx to peduncle. At 70 per cent R. H. the highest percentage of blossoms blighted from stamen infection, on the

eighth day after inoculation. Although invasion occurred readily by way of the stigma, the number of blighted blossoms was small.

In another experiment, the same procedure was followed except that inoculated floral parts were moved to the greenhouse as well as to controlled humidity cabinets after incubation for 10 hr. at 20° C. In the greenhouse and in 70 per cent R. H., infection spread only from stamen inoculation. In 98 and 78 per cent R. H. blossoms blighted from stigmatic as well as from stamen inoculation. Stamen inoculations gave the highest number of blighted blossoms; stigmas the next highest.

Small-flowered and large-flowered varieties[3] of peach were used in experiments to determine the liability of blossoms to infection in different stages of development. The former type has short, ovate petals; as the flower opens, the pistil and anthers become exposed before the petals separate. The latter type has showy large petals, more or less loosely folded in unopened stages, which cover the stamens and pistil until the blossom opens (Fig. 3, A).

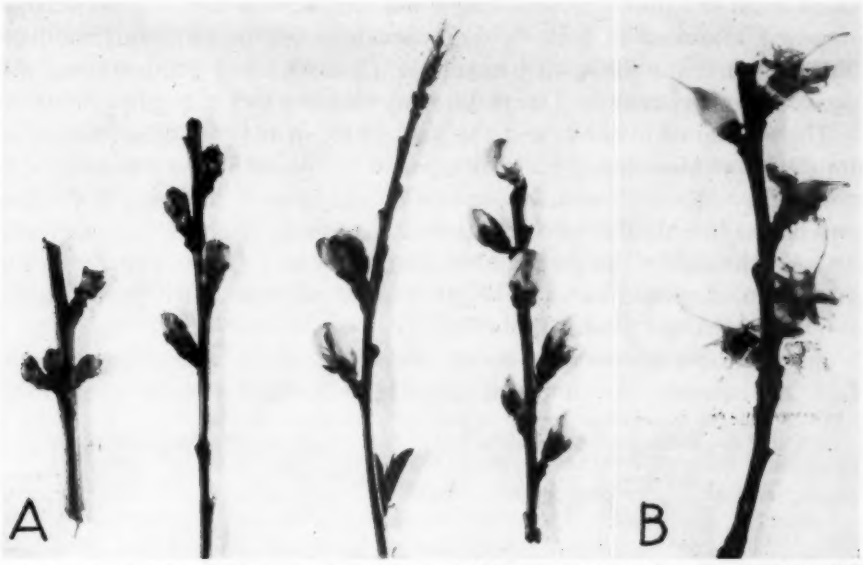

FIG. 3. A. Shoots from two types of peach blossom: left pair, small-petaled; right pair, large-petaled. B. Diseased shucks on young peach fruits. Inoculation made 12 days after pollination.

Open blossoms of both small- and large-flowered types are more liable to infection than unopened ones. Records were taken from blossoms removed to the greenhouse after an incubation period in saturated atmosphere (Table 4). Blossoms in various stages were inoculated with a spore suspension. Noninoculated blossoms remained free of disease and produced fruit.

[3] Four varieties of peach were available for greenhouse studies, namely: South Haven, Golden Jubilee, Valiant, and an unnamed seedling. The first three had small, ovate petals; the fourth had showy, large ones.

TABLE 4.—*Incidence of infection by Sclerotinia fructicola of peach blossoms inoculated in different stages of development*

Conditions of inoculation			Percentage of blossom blight from inoculation of	
Time of test	Period of wetness: Time, hours	Period of wetness: Temperature, ° C.	Open stage	Unopened stage
		Small-flowered varieties		
April 1940	24	25	100 (21)[a]	0 (20)
April 1941	24	25	85.7 (28)	1.7 (59)
February 1942	9	20	100 (14)	0 (11)
March 1942	15	20	100 (13)	0 (42)
		Large-flowered variety		
March 1942	23	15	75.4 (61)	4.3 (257)

[a] Figures in parentheses indicate the number of blossoms used.

The effect of higher relative humidities upon the incidence of infection of unopened or "pink" blossom stages was studied. A higher percentage of unopened blossoms of both flower types blighted in relative humidities above 80 per cent. Unopened small peach blossoms had petal, stamen, and stigma infection; unopened large-flowered blossoms had only petal infection.

The avenue of invasion and the percentage of blossom blight following inoculation of blossoms of the two types in unopened stages was studied in more detail. Each blossom was carefully tagged as to its stage of development before inoculation. After a period of wetness, the trees were moved to controlled humidity chambers and to the greenhouse. A high percentage of blossoms in unopened varieties blighted at 90 per cent R. H. from inoculation of stamens and pistil (Table 5).

Several experiments were conducted to determine when the enlarging fruit was past the danger of infection from diseased blossom parts (Fig.

TABLE 5.—*Percentage of blossom blight resulting from inoculation of blossoms of two varieties under different environmental conditions*

Blossom stage	Percentage of blossom blight at 90 per cent R. H.	80 per cent R. H.	40–100 per cent R. H.
	Small-flowered variety (Valiant)		
Open	100 (23)[a]	100 (3)	25 (4)
Unopened			
(a) Stamens or pistil exposed	73.3 (15)	12.5 (8)	57.1 (7)
(b) petals only	9.1 (11)	0.0 (33)	0.0 (6)
	Large-flowered variety (Seedling)		
Open	100 (2)	20 (10)	16.7 (12)
Unopened			
(a) balloon	60 (3)	.....	0.0 (9)
(b) less advanced	4.1 (49)	0.0 (49)	4.5 (22)

[a] Figures in parentheses indicate the number of blossoms used.

3, B). Peach blossoms were pollinated by hand soon after they were open. In preliminary studies, peach blossoms were inoculated by atomizing conidia upon them 2 days after pollination. After incubation for 24 hr. at 25° C., the blossoms were moved to the greenhouse, where over 75 per cent of them blighted after 7 days.

Blossoms inoculated thus ½, 2, 4, and 6 days after hand pollination blighted 34.3, 19.0, 7.7, and 0 per cent, respectively. The blossoms were moved to the greenhouse after 24 hr. in saturated atmosphere at 15° C. After development of the fertilized ovary for 4 days, less than one-tenth of the blossoms with enlarging fruit blighted. Although infection occurred on the floral parts, discoloration and rot did not spread past the calyx. After development of the fertilized ovary for 6 days prior to inoculation, infection occurred only on stamens and pistils, since the petals had fallen. There was no loss of fruit; the discolored shuck became dry and separated from the peduncle on the fourth day after inoculation (the tenth day after pollination).

In similar experiments, a very small loss of fruit occurred when blossoms were inoculated 5 days or more after pollen was placed on the stigma. Where normal growth of the fertilized ovary occurred, the discoloration did not spread past the calyx. Although infection occurred in the pistil, the disease did not spread into the ovary from the style. Pure cultures of *S. fructicola* were obtained from the brown styles and blossom remnants (shuck) on the small fruits. Blossoms that had not been fertilized or were too weak to develop fruit in a normal manner blighted.

The effect of relative humidity was studied on the incidence of blossom blight following inoculation of blossoms, with small fruit of different ages. A spore suspension was atomized on fruit in three different stages of development. Small- and large-flowered varieties were incubated for 15 hr. at 15° C. before removal to 70, 80, and 90 per cent R. H. chambers. Fruit, 11 days after pollination, was almost at the size to separate the calyx from peduncle; fruit, 6 days after pollination, almost filled the calyx cup; the third stage was blossoms 1 day after pollination. At 90 per cent R. H. there was appreciable loss of fruit inoculated 11 days after pollination (Table 6). These fruits were destroyed by the rotting of the pedicel. Approximately 50 per cent loss of fruits of both flower types occurred when inoculated 6 days after pollination. At 80 per cent R. H. there was negligible loss of fruit 6 and 11 days old; discoloration did not spread beyond the calyx. There was almost complete loss of blossoms when inoculated 1 day after pollination. At 70 per cent R. H. some blighting was recorded on those blossoms with 6 days of growth after pollination. Usually, however, as the relative humidity was lowered, the diseased blossom parts became dry and pedicel infection did not occur.

Forty hours in a saturated atmosphere at 19° C. was considered the minimum period for pedicel infection of young peach fruits 9 days after pollination. The calyx was enclosing the young fruit but was free from its

basal attachment. Inoculation was made by atomizing fruits and "shuck" with conidia. Invasion of the enlarging peach above the diseased pedicel was not observed. Fruits of the Montmorency cherry in the shuck-slip stage of development were inoculated and submitted to the same periods of saturation. Direct invasion and rotting of the sour cherry fruit from the enclosing freed shuck took place after 26 hr. and longer. Rot was not observed on larger, uninjured green cherry fruit where the shuck had already dropped before inoculation. Fruit which failed to develop normally, blighted.

DISCUSSION

Germination of conidia of *S. fructicola* on peach petals at 5° and 10° C. within 12 and 7 hr. respectively indicates that low temperatures would not prevent infection in the orchard under conditions of abundant inoculum and moisture. Roberts and Dunegan (24) produced typical symptoms of blos-

TABLE 6.—*Relation of relative humidity to the incidence of infection by S. fructicola on post-bloom stages of development*

Relative humidity and temperature	Time after pollination (days)	Percentage of loss	
		Small-flower type	Large-flower type
90 per cent, 20° C.	1	100.0	100.0 (19)[a]
	6	53.5 (15)	42.8 (14)
	11	47.0 (17)	13.6 (22)
80 per cent, 20° C.	1	100.0	70.6 (17)
	6	0.0 (14)	0.0 (15)
	11	4.1 (24)	0.0 (27)
70 per cent, 15° C.	1	25.0 (16)	55.5 (18)
	6	0.0 (16)	25.0 (20)
	11	0.0 (13)	0.0 (25)

[a] Figures in parentheses indicate the number of blossoms used.

som blight as a result of inoculating stigmas of peach with conidia and ascospores of *S. fructicola*. Ascospores, which also may cause blossom infection, were not used in the present studies and temperature studies of germination have not been seen. The optimum range for germination of conidia in all media was 20° C. to 25° C., although germination occurred in a relatively short time at 15° C. on potato-dextrose agar and on peach petals.

Wellman and McCallan (31) reported the optimum range for germination of conidia of *S. fructicola* on glass to be 21° to 27° C. Using LD50 values, they found a temperature effect at 10° C. and 35° C. when fungicidal compounds were not rated in the same order as at the optimum temperatures. Also, McClellan (21) noted that the toxic materials copper and sulphur on glass slides are least effective at the optimum temperature for *S. fructicola*. Data in table 1 indicate that the suscept is a much better medium for germination studies at low temperatures. It might be expected that larger dosages of a fungicide are necessary in the orchard at

temperatures below the optimum than would be indicated from laboratory assays on glass.

Precipitated moisture is necessary for germination of conidia of *S. fructicola* on glass and on most surfaces of flower parts. Dry conidia germinated on the anthers and on the stigma at 96 per cent R. H. and on the stigma at 80 per cent. Later experiments showed the stamens and pistil to be in many cases the infection courts for blossom infection. After inoculation with a spore suspension, penetration of all floral organs occurred. By restricting the inoculum to only one floral organ and immediately placing the blossoms in humidity chambers, it was possible to show that the fungus can eventually involve the entire blossom from each infected organ only at relative humidities above 90 per cent. After inoculation and an incubation period in the moist chamber, high percentages of blossoms blighted at 70 per cent R. H. and above. The majority of peach blossoms blighted from inoculation of stigma and stamens. From initial infections on stamens the fungus rapidly spread through the mass of stamens into the calyx cup. Blossoms blighted severely from stigmatic infection when held above 80 per cent R. H. Although infection of the stigma invariably occurred, the number of blossoms blighted by *S. fructicola* was less than from stamen infection. The fact that anthers and stigma are infection courts is important with regard to timing of protective sprays, since on many varieties these organs are exposed to inoculum before the blossoms open, exposing the total petal surface (Fig. 3, A). In high humidities, sporodochia of the fungus first appeared on the anthers. In 1948 Calavan and Keitt (3) reported, for *Sclerotinia laxa* on sour cherry, that "blossom infection originates in anthers more often than in any floral part."

Data presented indicate that *S. fructicola* infected blossoms in a saturated atmosphere within 18 hr. at 10° C., 8 to 12 hr. at 15° C., 11 hr. at 20° C., and 5 hr. at 25° C. These data refer to blossoms removed to greenhouses (40 to 100 per cent R. H.) after a period in saturated atmosphere. After removal of blossoms from a saturated atmosphere to controlled humidities, the greatest percentage of blossom blight always occurred at high relative humidities.

Diseased spots failed to enlarge (and sporodochia of the fungus failed to develop) when infected blossoms were held in 80 per cent R. H. or below. It is possible that under natural conditions dry weather would check the rotting of flower parts so that the diseased "shuck" will be abscised as the fruit enlarges.

It was noted that many weak, small blossoms do not normally complete the development of fruit. Such weakened blossoms are very susceptible to infection by *S. fructicola*. It is also possible that frost-injured blossoms are readily blighted.

In a favorable environment, *S. fructicola* often grows into the twig from diseased blossoms. In this study, gumming and browning of tissues was observed about 10 days after inoculation. A high moisture content in the

medium is requisite for continued growth of the hyphae of *S. fructicola*. *In vitro*, growth of this fungus was uninterrupted at relative humidities above 96 per cent and was retarded below 93 per cent. Apparently the moisture content of the twigs and their environment determines whether cankers are formed from diseased blossoms. Further, although the fungus has been isolated from overwintering cankers, the tissues may be too dry for spore production at blossom time the following season. Willison (33) reasoned that although *Sclerotinia* is found in wound-gum-impregnated wood, tissues are too dry for steady growth.

Two types of peach blossoms were used for infection studies; namely, small flowers with short ovate petals, and showy large-petaled blossoms. In the small-petaled type the stigma and anthers became exposed in the "pink" stages before the petals separated; in the large-petaled blossoms the petals covered the stamens and pistil until the open stage began. On trees removed to the greenhouse after inoculation, blossoms of both types that were open blighted, while blossoms in the pink stages had less than 15 per cent blight. Peach blossoms only partly opened were not easily infected, and some escaped infection in tests conducted by Roberts and Dunegan (24). To determine the percentage of blossom blight and the avenue of invasion of the pathogen into the pink stages of the two blossom types, blossoms were inoculated and after a period in a saturated atmosphere were moved to controlled humidities. It is of interest that in the small-petaled variety under humid conditions (90 per cent), only those blossoms in pink stages blighted in large numbers where the pistil or stamens were exposed. In the large-petaled type, the advanced pink stages were open sufficiently to permit inoculation and infection of the stamens and pistil at 90 per cent R. H. Considerably less blighting occurred in the less advanced pink stages (Table 5). It is evident, therefore, that under relatively low humidity, blossoms in pink stages with petals only exposed did not blight. At high humidities infection and blight occurred, especially when the stamens and pistils were exposed.

Several experiments were conducted to determine at what stage of the young developing fruit the danger from blossom infection is past. Peach blossoms were pollinated on opening and were left for various periods of time for fruit to develop before inoculation with conidia. A period of 24 hr. in a saturated atmosphere at 15° C. was given after inoculation, before the trees were moved to the greenhouse. Lesions appeared in all floral parts of blossoms inoculated on the third to the tenth day after pollination. The percentage of blight and loss of young fruit was negligible, however, after 5 days had elapsed between pollination and inoculation (Table 6). The fungus did not spread into the fruit or peduncle because of the abscission of the calyx from the peduncle and desiccation of the floral organs. To determine the percentage loss of young fruit when periods longer than 24 hr. in a saturated atmosphere were given after inoculation, several experiments were conducted. The first evidence of fungal invasion of the small green

fruits, 9 and 12 days after pollination, was peduncle rot which occurred after approximately 40 hr. in a saturated atmosphere at 19° C. However, Montmorency cherry fruits of the same age as the peaches were directly invaded from the enclosing freed calyx after a period of 26 hr. in a saturated atmosphere. In all cases, unfertilized fruits readily blighted.

SUMMARY

Conidia of *Sclerotinia fructicola* germinated at 5° C. to 30° C. Optimum temperatures were 15° C. to 25° C. At low temperatures of 5° C. and 10° C., germination occurred in a much shorter time on peach petals than on glass slides.

Radial growth of *S. fructicola* on potato-dextrose agar was most rapid at 20° C. to 25° C. Some growth occurred at 30° C., but none at 35° C. Sporulation occurred in 10 days at 5° C., in 8 days at 10° C., in 3 days at 15° C., and in 2 days at 20° C. and 25° C.

Conidia of *S. fructicola* dusted on petals, stamens, and stigmas germinated on all organs at 100 per cent R. H., on stamens and stigmas only at 96 per cent, and on stigmas only at 80 per cent. Maximal radial growth on potato-dextrose agar at 25° C. occurred at 96 per cent R. H. equivalent and above.

Sclerotinia fructicola infected blossoms in a saturated atmosphere within 18 hr. at 10° C., 8 to 12 hr. at 15° C., 11 hr. at 20° C., and 5 hr. at 25° C.

Blossoms blighted by way of all the floral organs at 90 per cent R. H. At 80 and 70 per cent R. H., blight occurred from infected stigmas only. When short periods in a saturated atmosphere followed inoculation, blossoms blighted from infection of all floral organs, with the exception of sepals, on removal to humidities above 80 per cent. At 70 per cent R. H. in the greenhouse, blighting of blossoms occurred only from stamen infection.

Open blossoms of small- and large-flowered peach blossoms are more liable to infection than unopened buds. However, unopened blossoms blighted at high relative humidities. Infection occurred on exposed stamens and stigma.

The percentage of blossom blight and loss of young fruit was negligible when 5 days had elapsed between pollination and inoculation. After abscission of calyx, the uninjured fruits are not liable to infection by *S. fructicola*. However, pedicel infection of individual fruit, 11 days after pollination, occurred at 90 per cent R. H. Cherry fruits of the same age were directly invaded from the enclosing freed calyx.

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PHYTOPATHOLOGICAL NOTES

Two, 4-D Effects Can Persist in Plant Tissues. J. G. Brown. Among numerous plants injured by the ester form of 2,4-D in Arizona are cotton, grape, cantaloupe, trumpet vine (*Campsis radicans*), and Melia. The last-named is unusual in showing persistence of the effects of the weedicide.[1]

Two, 4-D was applied to a weed-infested lawn under trees of *Melia azedarach* L. in Tucson just before autumnal leaf-fall in 1948. Grass and weeds were thoroughly wetted with the weedicide in a concentration of 2 tablespoonfuls of the ester form of the chemical per gal. of water. No symptoms of injury to the trees were evident until, in the following spring (1949), months after the weedicide had been applied to the lawn, the new foliage had characteristic effects of the chemical. Leaves were dwarfed and chlorotic; leaflets ranged from near normal size to mere vestiges; some secondary leaflets had abnormal, fringed margins.

To one familiar with the effects of 2,4-D on the foliage of susceptible plants, the symptoms described above were unmistakable. The weedicide had not been used by any resident of the immediate neighborhood in the spring of 1949, nor were Melia trees abnormal that are growing two blocks distant. Furthermore, trees of *Melia azedarach* throughout the city and under the observation of the writer for more than 40 years have had remarkably healthy foliage, certainly no diseased condition that even remotely resembles that described above.

Effects of 2,4-D are reported[2] to be nonpersistent in *Melia azedarach* in Texas. The authors of the report omit details of the concentration of the weedicide and the time of year it was employed.—Department of Plant Pathology, University of Arizona, Tucson, Arizona.

Phytophthora Blight of Safflower.[1] Gino Malaguti. In Maracay, State of Aragua, Venezuela, in extensive irrigated experimental plots[2, 3] of safflower (*Carthamus tinctorius* L.), a serious and apparently new disease was observed. Seed of two varieties, Spiny and Spineless, had been obtained from Dr. C. E. Claassen of the University of Nebraska.[3]

During the dry season (February) of 1949, the chief diagnostic symptoms of this disease were a rapid, permanent, progressive color change in

[1] Brown, J. G. Cotton, other plants, injured by 2,4-D. Ariz. Farmer (Phoenix) **28**: 4–5. July 23, 1949.

[2] Tullis, E. C., and W. G. Davis. Persistence of 2,4-D in plant tissues. Science **111**: 90. 1950.

the leaves from dark-green to light-green and, finally, death of the plants. A blackish-brown discoloration, turning reddish-brown when the plants dried, occurred on the lower stem area and extended downwards slightly and upwards for 15–20 cm. from the soil level. Spread was far more rapid vertically than horizontally. Roots appeared normal and were seldom infected. Diseased plants usually died, remaining erect, within 5–8 days after symptoms appeared. Plants sown in different periods of the season were infected at their blossom stage, the Spiny variety being far more susceptible than the Spineless.

During the rainy season (June) infection occurred at every stage of plant development and all parts were attacked. Seedlings and young plants were killed by a girdling infection at the base of the stem, which was water-soaked and discolored. A canker 5–15 mm. long caused the collapse of the stem and the plant then lodged. In older plants symptoms of collar rot were almost the same as those described for the dry-season plantings, except that the infected area was somewhat darker. Infection in aerial parts of plants (stems, branches, leaf stalks, flowers) caused death of the infected zone either by direct invasion or by destruction of conductive tissue. In woody plants the fungus usually spreads slowly, causing death by destruction of the lower stem tissues. A soft, water-soaked, blackish-brown decay finally involved all tissues of invaded roots.

A *Phytophthora* was consistently isolated from the advancing margin of decay in roots and stems of seedlings and from both young and mature plants. Malt extract, cornmeal, and potato-dextrose agars were satisfactory culture media. Mycelial growth was irregular and tufted on most media. Aerial hyphae, variable in width, richly granular, and sometimes knotted, were abundant. Sporangia formed profusely on cornmeal agar at room temperature (about 26° C.) in 5 days. They were irregularly ovate to ellipsoidal, sometimes almost spherical, and had prominent papillae. Length ranged from 27.25 to 46.75 μ and width from 18.10 to 28.96 μ. Average size was 34.5×23.9 μ but slightly larger ones were produced in 2 days in Petri's solution. Germination occurred by production of zoospores or by a germ tube.

Chlamydospores, averaging about 21.8 μ in diameter, were produced abundantly at about the same time as sporangia, and were especially prevalent in old cultures.

No oogonia or oospores were observed in 5-month-old cultures and they were not produced when cultures of various safflower isolates were paired

[1] Acknowledgment is made to Dr. W. F. Jeffers, University of Maryland, for assistance in preparing this manuscript.

[2] About 15,000 plants of each variety were planted by the Departamento de Genetica, Instituto Nacional de Agricultura, Maracay, Venezuela.

[3] "The Spineless variety of safflower which was increased in Venezuela has been designated as Nebraska No. 3; it is a selection from a Spiny variety known as "Special Russian." The Spiny variety, known as Nebraska No. 9, is a selection of an introduction from Khartoum, Anglo-Egyptian Sudan." (From a personal letter of Dr. Claassen to the writer.)

with each other or with *Phytophthora* sp. from sesame, *P. capsici*, or *P. parasitica* from tobacco. Amphigynous sexual organs, however, were found in all paired cultures of safflower isolates with *Phytophthora faberi* from cacao.

Pathogenicity was demonstrated for four safflower isolates and the re-isolated organism was identical with the original fungus. This organism was also pathogenic to tomato and cacao fruits and to zinnia seedlings. It failed to infect watermelon fruits and seedlings of papaw, tomato, pepper, and sunflower.

This *Phytophthora*, from its morphology, cultural behavior, and growth at critical temperatures, is referable to the group of *Phytophthora palmivora* Butler.[4, 5]

A survey of literature shows that no mention of a similar Phytophthora blight of safflower has been reported from Venezuela or elsewhere. However, a blight of potted safflower seedlings caused by *Phytophthora palmivora* has been reported in India[6] and an undetermined root rot of this host has been reported in the United States.[7]—Division de Fitopatologia, Instituto Nacional de Agricultura, Maracay, Venezuela.

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[6] Balakrishnan, M. S., and C. S. Krishnamurthy. Seedling blight of safflower, *Carthamus tinctorius* L. Curr. Sci. **16**: 291–292. 1947.

[7] Claassen, C. E., M. L. Schuster, and W. W. Ray. New diseases observed in Nebraska on safflower. U. S. Dept. Agr., Pl. Dis. Reptr. **33**: 73–74. 1949.

BOOK REVIEWS

Ainsworth, G. C., and Kathleen Sampson. *The British Smut Fungi (Ustilaginales).* 137 pp. 21 fig. 2 pl. The Commonwealth Mycological Institute, Kew, Surrey. 1950. 20s or $3.00. A discussion of the economic importance of British smut diseases precedes chapters on classification, biology, cytology, genetics of smuts, techniques, and British smut fungi. A bibliography lists 610 papers.

Bawden, F. C. *Plant Viruses and Virus Diseases.* 3rd rev. ed. 335 pp. 59 fig. Chronica Botanica Co., Waltham, Mass. Stechert-Hafner, Inc., New York. 1950. $6.00. Taxonomy, symptomology, and methods of transmission; relations of viruses and vectors, virus disease and host-plant physiology, and control measures; methods of assay, serology, purification, and crystallinity of plant viruses; the chemical and physical properties of purified viruses; particle size; types of inactivation; and speculations on the origin of viruses comprise the 17 chapters of this edition.

Padwick, G. Watts. *Manual of Rice Diseases.* 198 pp. 49 fig. The Commonwealth Mycological Institute, Kew, Surrey. 1950. 30s or $4.50. Part I has chapters on seedling blight and root rot, diseases of foliage, stem and leaf sheath, grain and inflorescence, and diseases caused by nematodes. Part II includes diseases resulting from viruses, deficiencies, and unfavorable soil conditions. Part III is an annotated check list of 232 species of fungi found on rice. In all, 38 diseases are described.

Chitwood, B. G., and M. B. Chitwood. *An Introduction to Nematology.* Section I, Anatomy. Rev. ed. 213 pp. 145 fig. Box 104, Catholic University, Washington, D. C. 1950. $10.00. A discussion of the general structure of nematodes is followed by 12 chapters covering details of tissues and organs that compose the body systems; last, a chapter on nemic relationships. Many detailed drawings accompany the text.

Buller, A. H. Reginald. *Researches on Fungi.* Vol. VII. 458 pp. 124 fig. University of Toronto Press, Toronto. 1950. $13.50. Editor G. R. Bisby made practically no alteration in the 12 chapters dealing with the sexual process in the Uredinales. Described in this volume are the discovery of the function of pycnidia, the protoaecidia, flexuous hyphae, the union of pycnidiospores and flexuous hyphae in four rusts, modes of initiating the sexual process, comparison of sex in Uredinales and Hyphomycetes, cytology of the sexual process in heterothallic Uredinales, sex in *Cronartium ribicola* and in *Puccinia suaveolens*, the genus Gymnosporangium, and the evolution of pycnidia.

Maneval, Willis E. *A supplementary list of Missouri fungi* (with special reference to plant pathogens and wood-destroying fungi). 65 pp. University of Missouri Studies, Vol. 22, No. 3. University of Missouri, Columbia, Mo. 1948. $2.00.

Proceedings of the Jubilee Meeting (British Mycological Society) held in London, 20–25 October, 1946. 208 pp. 38 fig. 8 pl. 1948. Cambridge University Press. 40s. Agents for United States, University of Chicago Press, Chicago, Ill. $10.00. Fifteen papers of general biological significance edited by B. Barnes and W. D. Moore.

Conservacion de Granos y Almacenamiento en Silos Subterraneos. [Conservation of grain and storage in underground silos.] 222 pp. 75 illus. 3 pl. Memoir prepared by Service of Subterranean Silos, Minister of Agriculture and Livestock. Buenos Aires, Argentina. 1949. Written in Spanish.

Advances in Agronomy. Vol. 1. 439 pp. Academic Press, Inc., New York. 1949. $7.50. Edited by A. G. Norman, this volume contains 13 reviews on fertilizers, soil minerals, boron in soils and crops, fixation of phosphorus, growth in saline and alkaline soils, microorganisms and roots, weed control, and agronomy of alfalfa, potatoes, and soybeans.

Iodine and Plant Life. 114 pp. Chilean Iodine Educational Bureau. Stone House, Bishopsgate, London EC2. 1950. A review of literature on the iodine content of microorganisms, seaweeds, and land plants, plus the role of iodine in growth and development, and in plant protection, is followed by an annotated bibliography of 784 papers. Available without charge to agricultural workers in the United States and Canada, from Chilean Iodine Educational Bureau, Inc., 120 Broadway, New York 5.

Bonner, James. *Plant Biochemistry.* 537 pp. 124 fig. Academic Press, Inc., New York. 1950. $6.80. Thirty chapters include topics on carbohydrates and carbohydrate metabolism, cell walls and cell-wall metabolism, plant acids and plant respiration, metabolism of nitrogen compounds, secondary plant products, and certain aspects of plant growth.

Kelly, A. P. *Mycotrophy in Plants.* 223 pp. 20 pl. and illus. Chronica Botanica Co., Waltham, Mass.; Stechert-Hafner, Inc., New York. 1950. $4.50. Included are 12 chapters on the biology of mycorrhizae and fungus-infected rhizomes and thalli—also the taxonomy, ecology, and physiology of mycorrhizae.

Stebbins, G. Ledyard, Jr. Variation and Evolution in Plants. 643 pp. 55 fig. Columbia University Press, New York. 1950. $8.00. This is a general work having topics that range from isolation and origin of species to genetic systems as factors in evolution. The role in evolution of variation patterns, apomixis, hybridization, polyploidy, and fossils is thoroughly explored, and from this the evolutionary trends in plants are presented.

NATIONAL SCIENTIFIC REGISTER

Within the next few months, approximately 150,000 American scientists and technologists will be asked to participate voluntarily in one of the most comprehensive registrations of scientific personnel yet attempted by the Government. Final plans have been completed for this operation by the National Scientific Register, sponsored by the National Security Resources Board, and administered by the Office of Education, Federal Security Agency. The registration will include qualified scientists in the natural and physical sciences, and will be accomplished through the direct assistance of the principal professional and scientific societies. The purpose of the registration is to provide urgently needed information on the country's supply of technological personnel in the many scientific fields—information needed for both immediate and long-range mobilization planning.

The need for an analytical index of American scientists became apparent to the NSRB when lack of specific information began to hamper seriously efforts to prepare complete and comprehensive mobilization plans. Provision has been made for such a register in legislation creating the National Science Foundation. However, because of the international situation early in 1950, the NSRB decided that this important function could not be delayed longer, and that some interim action should be taken pending the creation of the NSF. Accordingly, the National Scientific Register was established in the Office of Education in the summer of 1950. James C. O'Brien of the Office of Manpower, National Security Resources Board, who formerly headed the wartime National Roster of Scientific and Specialized Personnel, was named Director of the new enterprise.

The National Scientific Register was given the responsibility for providing answers to two pressing questions in the over-all manpower problem: 1) What are the characteristics of the supply of scientific and technological manpower in the country? and 2) What will be the requirements for scientists and technologists in all phases of mobilization?

It appeared that the only practical method of obtaining answers to the first of these questions was to effect a voluntary registration of scientists by means of questionnaires designed to obtain the specific types of information required. Committees of leading scientists in each of the fields to be covered were consulted and an effort was made to develop specialty check lists which would serve as the basis for all of the various types of studies required.

The scientific fields to be included in the present registration are as follows: physics, chemistry, the biological sciences, geology, geophysics, meteorology, astronomy, mathematics, and crystallography. Registration will not be made in the engineering fields at this time since the recent study of American engineers, sponsored by the Office of Naval Research and completed under the direction of the Engineers Joint Council, has been made a part of the National Scientific Register.

Information obtained through circularization of scientists will be analyzed and interpreted by a special coding group, and the results will be recorded in an electronic punch card system. A survey was made of the various newly developed techniques for the mechanical recording, tabulating, and interpreting of such data, and the Register has adopted several which should result in the most advanced system of personnel registration developed.

The National Scientific Register project has been given a high priority by the NSRB in view of the critical role played by American science in the last war, and in recognition of the fact that American security and supremacy in a future war will depend to an important degree on the total utilization of scientific and technological skills. For this reason, it is considered important that every scientist who is circularized in the current registration, return his questionnaire as quickly as possible. Important statistical studies need to be undertaken at an early date, and the comprehensiveness of data included in these studies will be an important factor in the total mobilization planning effort of the Government. While the National Scientific Register at present does not envision a large-scale placement function, data obtained from individual scientists will be recorded in such a manner that the system will lend itself to whatever type of placement or distribution program might be required in an all-out mobilization effort, should one occur.

October, 1950

Federal Security Agency
Office of Education
Washington 25, D. C.

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Page 950, line 13, *delete* (Fig. 1).
Page 984, table 3, line 1, *read* 6 p. m. to midnight *for* 8 p. m. to midnight.
Page 1055, footnote 5, *read* Schwarze for Schwartz.

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Page 100, last four lines, *read:*
are thin-walled and therefore thought to be of *H. sacchari.* According to Martin (13, p. 64), Matsumoto and Yamamoto in 1935 proposed the name *Cochliobolus stenospilus* for the perfect stage of an *Helminthosporium* identified as *H. stenospilum,* which they obtained in pure culture. The com- . . .
Page 296, line 2, *read* concerned *for* cencerned.
Page 297, Literature Cited, reference 9, *add* 1926.
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Seed Treating News

from DU PONT

TREATING CERTIFIED SEED

by Julian H. Miller—University of Georgia

(This article is reprinted by permission of the author from the "Georgia Crop Improvement News", Volume II, Number 8.)

We find that many growers are under the misapprehension that if seed are certified, they do not need to be treated. We have found in the laboratory over a period of many years that even the best certified seed are carriers of disease-producing organisms, and substantial increases in stands and yields can be secured by proper seed treatment. These results are obtained not only by surface sterilizing the seed, but due to the presence of the disinfectant, a sterile area is formed around each seed in the soil, so soil organisms cannot immediately attack the seedling.

Follow the recommendations of your County Agent or manufacturer and treat certified seed before planting.

Barley, Oats and Wheat—all of these small grains carry disease-producing fungi on the seed coats, and increased stands and less smut can be secured by treating with a disinfectant. This is especially important with oats and barley that are subject to attack by the *Helminthosporium fungi.* It is well to remember that rusts and powdery mildews produce spores that blow into the fields through the air, and that control is possible only through the use of varieties that are disease-resistant.

Dust Treatment: N. I. "Ceresan"*—½ oz. per bushel. *Slurry Treatment:* "Ceresan" M—1 lb. per gallon of water.

Grasses—All grass seed should be treated, especially Ky 31, and Alta fescue, as species of *Helminthosporium* come in on the seed. We are now bringing in new grasses and developing pastures, so this is very important.

Dust Treatment: "Arasan"—8 ozs. per 100 lbs. of seed. *Slurry Treatment:* "Arasan" SF—3 lbs. per gallon of water.

* Ed. Note: "Ceresan" M is replacing New Improved "Ceresan". It is used at the same rate, is equally effective and easier to use.

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